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REVIEW ARTICLE

# ABCC2/Abcc2: a multispecific transporter with dominant excretory functions

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**Abstract**

ABCC2/Abcc2 (MRP2/Mrp2) is expressed at major physiological barriers, such as the canalicular membrane of liver cells, kidney proximal tubule epithelial cells, enterocytes of the small and large intestine, and syncytiotrophoblast of the placenta. ABCC2/Abcc2 always localizes in the apical membranes. Although ABCC2/Abcc2 transports a variety of amphiphilic anions that belong to different classes of molecules, such as endogenous compounds (e.g., bilirubin-glucuronides), drugs, toxic chemicals, nutraceuticals, and their conjugates, it displays a preference for phase II conjugates. Phenotypically, the most obvious consequence of mutations in ABCC2 that lead to Dubin-Johnson syndrome is conjugate hyperbilirubinemia. ABCC2/Abcc2 harbors multiple binding sites and displays complex transport kinetics.

**Keywords:** *ABCC2; MRP2; ABC transporter; Dubin-Johnson syndrome; conjugate hyperbilirubinemia; pharmacokinetics; ADME; toxicity*

**Abbreviations:** ABC: ATP-binding cassette transporter; ABCB1: also known as P-glycoprotein (P-gp) or Multidrug resistant protein 1 (MDR1); ABCB11: bile salt export pump (BSEP); ABCC2: multidrug resistance associated protein 2 (MRP2, also known as c-MOAT); BDL: bile duct ligation; BGB: bisglucuronosylbilirubin; CDCF: carbodichlorofluorescein; CMV: canalicular membrane vesicles; DJS: Dubin-Johnson Syndrome; DNP-SG: dinitrophenyl-glutathione; EHBR: Eisai-hyperbilirubinemic rats; E2-3bG: estradiol-3β-glucuronide; E2-17bG: estradiol-17β-glucuronide; GY: Groningen Yellow, transport deficient rat strain; HEK: human embryonic kidney cells; LTC4: leukotriene-C4; MDCKII: Madin-Darby canine kidney cells; MDR: multidrug resistance; MGB: monoglucuronosylbilirubin; MTX: methotrexate; PDZ: PSD95/Dlg/ZO1 binding motif; PhIP: 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine; SD: Sprague-Dawley rat; Sf9: Spodoptera frugiperda 9 insect ovarian cells; TCDC-S: taurochenodeoxycholate-3-sulfate; TLC-S: taurolithocholate-3-sulfate; TM: transmembrane; TMD: transmembrane domain; TR-: transport deficient rat strain; VT: vesicular transport.

## Introduction

It is increasingly recognized that transporters play a major role in the pharmacokinetics of drugs. They play a pivotal role in the barrier penetration of low passive permeability and/or low-solubility drugs (Wu and Benet, 2005). Uptake transporters mediate cellular uptake, and efflux transporters actively pump drugs out of cells. Polarized epithelial and endothelial cells express multiple transporters at high levels. Depending on the localization of the uptake and efflux transporters, the vectorial summation of their contribution is strikingly different. Uptake transporters may overcome the effect of the efflux transporters if both are located in the same membrane (Glaeser et al., 2007; Jain et al., 2008). On the contrary, uptake and efflux transporters cooperate when expressed in different membrane domains (i.e., apical vs. basolateral) of polarized cells (Cui et al., 2001; Sasaki et al., 2002). The most thoroughly studied models for cooperation of transporters are hepatocytes. An array of uptake transporters expressed in the basolateral/sinusoidal membranes and multiple ABC (ATP-binding cassette) transporters on the apical/

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canalicular membrane efflux endobiotics, xenobiotics, and their metabolites into the bile.

The ABCC (also known as multidrug-resistance–associated protein; MRP) subfamily of human ABC transporters is the largest subfamily, with 13 members (reviewed in Kruh and Belinsky, 2003). The majority of the proteins are active transporters, with a wide array of endobiotics and xenobiotics listed as substrates. The subfamily also contains examples of a channel, gated by ATP binding and hydrolysis (i.e., the cystic fibrosis transmembrane conductance regulator; CFTR/ABCC7) (Riordan et al., 1989) and regulators of ATP-dependent potassium channels, such as the sulfonylurea receptors, SUR1/ABCC8 and SUR2/ABCC9 (Inagaki et al., 1995, 1996) respectively). According to the predicted topology, ABCC1, ABCC2, ABCC3, ABCC6, ABCC9, ABCC10, ABCC11, and ABCC12 contain three membrane-spanning domains ($TMD_0$, $TMD_1$, and $TMD_2$), while ABCC4, ABCC5, ABCC7, ABCC8, and ABCC13 harbor only two membrane-spanning domains ($TMD_1$ and $TMD_2$). $TMD_1$ and $TMD_2$ are thought to have six and $TMD_0$ has six membrane-spanning helices (Deeley et al., 2006).

The subfamily members have been shown to be expressed in many tissues and cell types. At all physiological and pharmacological barriers, multiple members are expressed. In hepatocytes, four ABCC proteins are expressed at significant levels: ABCC3, ABCC4, and ABCC6 at the sinusoidal and ABCC2 at the canalicular membrane (Keppler, 2005).

ABCC2 (also known as cMOAT, MRP2) is a multispecific organic anion transporter expressed at important pharmacological barriers, such as the enterocytes, epithelial cells of proximal tubules in the kidney, and the canalicular membrane of hepatocytes, just to mention the most important ones. Among these sites, arguably, ABCC2 plays the most critical role in the canalicular membrane. At this location, it is involved in the biliary elimination of both endogenous and exogenous waste products—mostly as conjugates, as well as a variety of endogenous compounds and unmetabolized drugs.

This review will focus on the functional aspects of ABCC2. We will not cover regulation of ABCC2, as it will be covered by a separate review in this issue (see Manautou et al., in this issue).

## Cloning and structural features

The functional profiling of ABCC2 was aided by $TR^-$ (transport-deficient rat strain) rats, a mutant strain derived from Wistar rats (Jansenet al., 1985). These rats have an autosomal recessive transport defect, resulting in conjugated hyperbilirubinemia (Jansen et al., 1985). Subsequently, three other types of rats were identified: the Groningen Yellow (GY) rat (Kuipers et al., 1988) derived from the same colony as the $TR^-$, the Eisai-hyperbilirubinemic (EHBR) rat (Mikami et al., 1986; Yamazaki et al., 1995), and, more recently, the Lew.1w rats (Oswald et al., 2006b). When $TR^-$ were crossed with the GY (Kuipers et al., 1989) or with the EHBR (Kitamura et al., 1992), the pups were also hyperbilirubinemic, pointing to the same defect in all three types of rats. Canalicular transport of a variety of amphiphilic conjugates, such as bilirubin glucuronides, glutathione conjugates, and sulfate conjugates, were defective in these strains (reviewed in Paulusma and Oude Elferink, 1997b). Complementation of the genetic defect in EHBR rats, using adenovirus carrying the Abcc2 cDNA, corrected the canalicular transport deficiency (Hirouchi et al., 2005). The prior identification of ABCC1, then termed ABCC (Cole et al., 1992), has also aided in the identification of ABCC2. Primers, based on conserved domains of ABCC, were used to amplify the rat ortholog (Mayer et al., 1995). One of the products, a 347-bp (base pairs) cDNA, showed limited identity to the human ABCC. Moreover, this fragment could not be amplified from $TR^-$ liver mRNA, but only from the liver mRNA of wild-type rats. In addition, an antibody against a peptide corresponding to the 1,517–1,531 residues of ABCC-detected proteins, both in the sinusoidal and the canalicular membrane of the wild-type liver. In contrast, in the frozen sections of liver from the $TR^-$ rats, only sinusoidal staining was observed (Mayer et al., 1995). We now know that the antibody recognizes both ABCC1 and ABCC2.

The subsequent sequencing of rat cDNA (Buchler et al., 1996; Paulusma et al., 1996; Ito et al., 1997) revealed a nucleotide deletion in the ABCC2 gene of the $TR^-$ rats (Paulusma et al., 1996). The human cDNA was cloned the same year from cisplatin-resistant human cancer cell lines, where it was shown to limit the accumulation of drugs (Taniguchi et al., 1996) and, 1 year later, from human fibroblasts (Paulusma et al., 1997). Subsequently, cDNAs from many species were generated (reviewed in Konig et al., 2003). ABCC2 proteins from mammalian species most commonly used in drug research are highly homologous. The identities in protein sequence range from 77 to 87% (Konig et al., 2003).

The ABCC2 gene is organized into 32 exons in humans (Toh et al., 1999) and is localized on chromosome 10q24, as shown by fluorescent *in situ* hybridization (Taniguchi et al., 1996) (ACCESSION NG_011798). The genomic structure is similar to the intron-exon organization of ABCC1 (Grant et al., 1997).

The ABCC2/Abcc2 proteins have been expressed in functional forms in mammalian (Ito et al., 1998; Cui et al., 1999) and in insect cells (Madon et al., 1997; Bakos et al., 2000; van Aubel et al., 2002). The size of the protein from the pharmacologically most relevant species varies within a narrow range, with 1,541 amino acids of the rat protein as the shortest and 1,564 amino acids of the rabbit as the

longest (reviewed in Nies and Keppler, 2007). The human protein has 1,545 residues (Taniguchi et al., 1996).

According to the most widely used predicted structure, the 17 transmembrane (TM) segments of the three transmembrane domains ($TMD_0$, $TMD_1$, and $TMD_2$) of ABCC2 are organized as shown in Figure 1. The N-terminal domain, $TMD_0$, has about 200–280 amino acids, comprises five TM segments, and has an extracytosolic $NH_2$-terminus (Cui et al., 1999). The next TM segment, $TMD_1$, is connected to $TMD_0$ via a cytoplasmic loop ($L_0$). The $TMD_1$ and $TMD_2$ domains exhibit six TM segments each and are linked through an intracellular segment ($L_1$). The two cytosolic nucleotide-binding domains ($NBD_1$ and $NBD_2$) are localized toward the C-terminus from the respective TMD domain. $NBD_1$ is within the $L_1$ segment and $NBD_2$ is in the intracellular C-terminus of the protein. More recently, a predicted structure for ABCC2 was published that shows four transmembrane domains in $TMD_2$ (Nies and Keppler, 2007).

The most extensive analysis of the additional structural units ($TM_0$ and $L_0$) was performed in Madin-Darby canine kidney (MDCK II) cells, using deletion and complementation analysis (Fernandez et al., 2002). It was shown that $L_0\Delta$ABCC2, lacking $TM_0$, did not traffic to the apical plasma membrane and was associated with an intracellular compartment, likely endosomes. Coexpression with $TMD_0$, however, complemented $L_0\Delta$ABCC2, resulting in apical localization of both fragments. The fundamental role of the N-terminal domain in apical localization was confirmed by domain-swap experiments. When the $NH_2$-proximal region up to $TM_9$ of ABCC2 was exchanged with the corresponding region of ABCC1 (amino acids 1–480), the chimera localized to the basolateral membrane (Konno et al., 2003). Bandler (Bandler et al., 2008) further refined the analysis, showing that all elements necessary for apical targeting of ABCC2 reside in $TMD_0$ and the adjacent cytoplasmic loop. Further, they identified a lysine-rich element in the cytoplasmic loop that is essential for apical targeting.

Three putative *N*-glycosylation sites are predicted in ABCC2: amino acids 7 and 12 in the N-terminal domain and amino acid 1,011 between the putative TM segments, 12 and 13 (Fernandez et al., 2002). ABCC2, without the $TMD_0L_0$ domains ($\Delta$ABCC2), was only core glycosylated and localized intracellularly. In a complementation assay, coexpression of $TMD_0L_0$ with $\Delta$ABCC2 yielded full glycosylation of the protein and correct localization to the apical membrane (Fernandez et al., 2002).

Both $L_0\Delta$ABCC2 and $\Delta$ABCC2 had significantly lower stability than ABCC2. Coexpression of $TMD_0L_0$ with $\Delta$ABCC2 increased the stability of $\Delta$ABCC2. The degradation of $\Delta$ABCC2 was proteosome mediated, as proteosome inhibitors blocked degradation of the fragment (Fernandez et al., 2002).

The N-terminal domains are not sole determinants of localization and stability. The C-terminal domain has been also implicated in stability and correct localization to the apical membrane through protein-protein

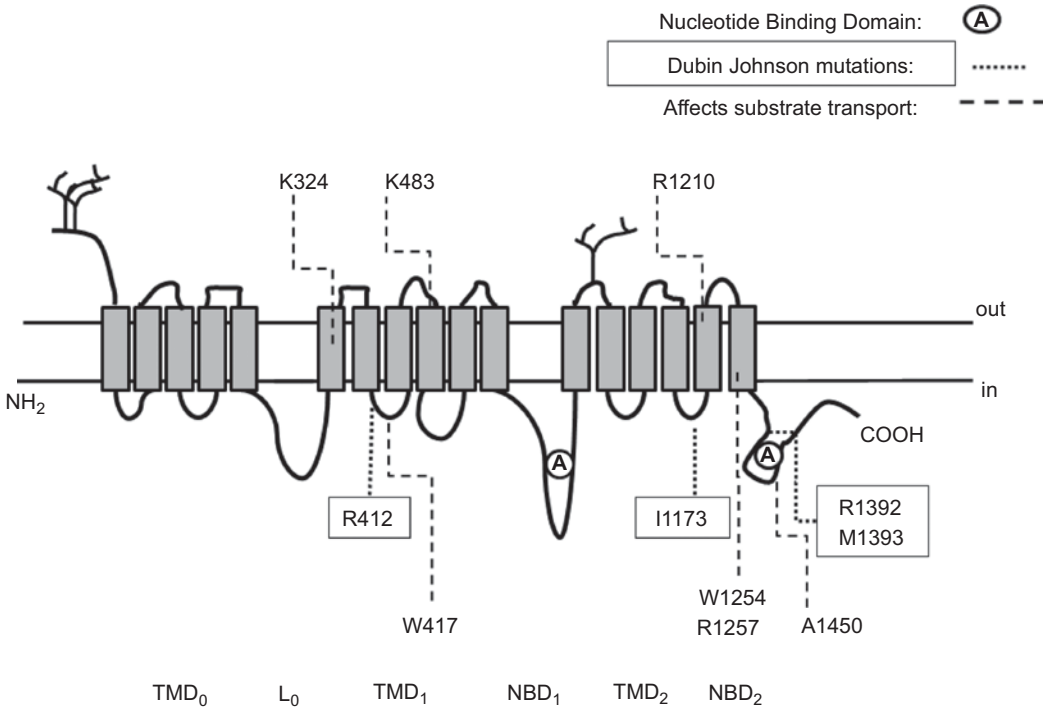


**Figure 1.** Schematic representation of the two-dimensional structure of ABCC2. Dubin-Johnson mutations (····) as well as amino-acid residues implicated in substrate transport (- - - ) are indicated. L0, linker domain; TMD, transmembrane domain; NBD, nucleotide-binding domain.

interactions. Radixin-deficient ($Rdx^{-/-}$) mice had lower levels of Abcc2, compared to other canalicular marker proteins, and developed conjugated hyperbilirubinemia (Kikuchi et al., 2002). Abcc2, via its C-terminal end, was shown to associate directly with radixin (Kikuchi et al., 2002), an adaptor protein known to cross-link integral membrane proteins with actin (Hoeflich and Ikura, 2004). Interestingly, ABCB1 also forms a complex with radixin in cells of lymphoid origin (Luciani et al., 2002), but was not downregulated in $Rdx^{-/-}$ mice (Kikuchi et al., 2002). Another study also found the very end of the C-terminus to be an apical targeting signal (Harris et al., 2001). As the TKF motif in the C-terminus meets the criteria of the PDZ (PSD95/Dig/ZO1)-binding motif (reviewed in Glynne and Evans, 2002), they predicted ABCC2 associations with PDZ proteins that were later shown experimentally by another group (Hegedus et al., 2003). However, other groups could not confirm a role for the C-terminal TKF motif in apical sorting of ABCC2, as neither the deletion of the last seven amino acids (Nies et al., 2002) nor the exchange of the C-terminal 51 amino acids for the corresponding ABCC1 sequence (Konno et al., 2003) affected apical localization of ABCC2. In addition, Abcc2 is correctly localized in $PDZK1^{-/-}$ mice (Kocher et al., 2003).

Several Dubin-Johnson mutations also affected ABCC2 trafficking, glycosylation, and stability. Deletion of Arg1392 and Met1393 leads to impaired maturation and trafficking of the protein from the ER to the Golgi complex (Keitel et al., 2000). The I1173F mutation showed a similar defect in trafficking (Keitel et al., 2003). Interestingly, two missense mutations, R768W and Q1382R, of the nucleotide-binding domains (NBDs) behaved differently. Pulse-chase analysis revealed that the precursor forms of the wild type and Q1382R ABCC2 fully matured, as they were resistant to endoglycosidase H (Endo H). However, the precursor form of the R768W remained sensitive to Endo H and was degraded within 120 minutes (Hashimoto et al., 2002). In all these cases, proteosome inhibitors blocked degradation of immature forms of mutant proteins (Keitel et al., 2000; Hashimoto et al., 2002; Keitel et al., 2003).

In conclusion, apical sorting of ABCC2 depends on multiple motifs and a correct tertiary, as well as quaternary, structure. Any mutation that affects correct protein folding will impair trafficking and, consequently, glycosylation and stability.

Residues and domains defining substrate specificity are coming from mutagenesis studies, including *in vitro* analysis of Dubin-Johnson variants (Ryu et al., 2000; Ito et al., 2001a, 2001b, 2001c; Fernandez et al., 2002; Hashimoto et al., 2002; Keitel et al., 2003; Hulot et al., 2005), studies from domain-swap experiments between ABCC1 and ABCC2 (Konno et al., 2003), as well as pharmacogenomic analysis of variants causing Dubin-Johnson syndrome (DJS) (reviewed in Nies and Keppler, 2007). All approaches are hampered by varying expression levels of the proteins and the difficulties to correlate transporter expression (Ito et al., 2001b). The mutants and the resulting phenotypes are listed in Table 1.

**Table 1.** Mutations determining substrate specificity.

| Mutant | Predicted location | Substrate | Activity changes | Reference |
|---|---|---|---|---|
| Human MRP2 | | | | |
| Δ1-188 | $TMD_0$ | LTC4 | ↓ | Fernandez et al., 2002 |
| K316A | JC, TM6 | GMF | ↔ | Ryu et al., 2000 |
| K324A | TM6 | GMF | ↓ | Ryu et al., 2000 |
| K329A | TM6 | GMF | ↔ | Ryu et al., 2000 |
| R412G DJ | IC | MTX | ↓ | Hulot et al., 2005 |
| W417I | IC, TM7-TM8 | E2-17βG | ↓ | Hirouchi et al., 2004 |
| | | LTC4 | ↓ | |
| | | DNP-SG | ↓ | |
| H439A | TM8 | GMF | ↔ | Ryu et al., 2000 |
| K483A | IC, JM, TM9 | GMF | ↓ | Ryu et al., 2000 |
| K590A | JC, TM11 | GMF | ↔ | Ryu et al., 2000 |
| S789F | NBD1 | E2-17βG | ↓ | Hirouchi et al., 2004 |
| | | LTC4 | ↓ | |
| | | DNP-SG | ↓↓ | |
| R1023A | EC, JM, TM13 | GMF | ↔ | Ryu et al., 2000 |
| H1042A | TM13 | GMF | ↔ | Ryu et al., 2000 |
| R1100A | JC, TM14 | GMF | ↔ | Ryu et al., 2000 |
| P1158A | IC, JM, TM15 | LTC4 | ↓↓ | Letourneau et al., 2007 |
| | | E2-17βG | ↔ | |
| | | MTX | ↔ | |

Table 1. continued on next page

**Table 1.** Continued.

| Mutant | Predicted location | Substrate | Activity changes | Reference |
|---|---|---|---|---|
| I1173F DJ | IC, TM15-16 | LTC4 | No act | Keitel et al., 2003 |
| | | E2-17βG | No act | |
| R1210A | EC, JC, TM16 | GMF | ↓↓ | Ryu et al., 2000 |
| R1230A | TM16 | GMF | ↔ | |
| R1257A | JC, TM17 | GMF | ↓↓ | |
| W1254A | JC, TM17 | E2-17βG | ↓↓ | Ito et al., 2001a |
| W1254C | | | ↓↓ | |
| W1254F | | | ↔ | |
| W1254Y | | | ↔ | |
| W1254A | JC, TM17 | LTC4 | ↓↓ | Ito et al., 2001b |
| W1254C | | | ↓↓↓ | |
| W1254F | | | ↓↓ | |
| W1254Y | | | ↓↓ | |
| W1254A | JC, TM17 | MTX | ↓↓ | Ito et al., 2001a |
| W1254C | | | ↓↓ | |
| W1254F | | | ↓↓ | |
| W1254Y | | | ↓↓↓ | |
| A1450T | NBD2 | E2-17βG | ↓↓ | Hirouchi et al., 2004 |
| | | LTC4 | ↓↓ | |
| | | DNP-SG | ↓↓ | |
| Rat Mrp2 | | | | |
| K308M | IC, JM, TM6 | TLC-S | ↔ | Ito et al., 2001b |
| | | DNP-G | ↑ | |
| | | LTC4 | ↓ | |
| | | E3040G | ↔ | |
| K320M | TM6 | TLC-S | ↑ | |
| | | DNP-G | ↑ | |
| | | LTC4 | ↓ | |
| | | E3040G | ↑ | |
| K325M | TM6 | TLC-S | ↓* | |
| | | DNP-G | ↓↓↓* | |
| | | LTC4 | ↓↓↓* | |
| | | E3040G | ↓ | |
| D329N | TM6 | TLC-S | ↔ | |
| | | DNP-G | ↓ | |
| | | LTC4 | ↓↓↓* | |
| | | E3040G | ↓ | |
| R586L | TM11 | TLC-S | ↓ | |
| | | DNP-G | ↓↓* | |
| | | LTC4 | ↓↓* | |
| | | E3040G | ↔ | |
| R1019M | IC, JM, TM13 | TLC-S | ↔ | |
| | | DNP-G | ↑* | |
| | | LTC4 | ↔ | |
| | | E3040G | ↔ | |
| R1096L | TM14 | TLC-S | ↑ | |
| | | DNP-G | ↑ | |
| | | LTC4 | ↔ | |
| | | E3040G | ↔ | |

EC, extracellular; IC, intracellular; JC, near the cytosol in the membrane; JM, juxtamembrane; TLC-S, tauro-litocholate-sulfate; GMF, glutathione-methyl-fluorescein; ↑, activity over control>1.2; ↔, 1.2>activity over control>0.8; ↓, 0.8>activity over control>0.5; ↓↓, 0.5>activity over control>0.1; ↓↓↓, 0.1>activity over control.
*Statistically significant.

The protein structure-activity correlations are complex, as multiple residues or domains seem to determine substrate transport. Mutagenesis studies of human ABCC2, replacing basic residues in the TM as well as in the intracellular or extracellular juxtamembrane domains with alanines, showed that residues in $TM_6$ (K324) and $TM_{17}$ (R1257), a residue in the intracellular loop in proximity of $TM_9$ (K483), as well as a residue in the extracellular loop in the close proximity of $TM_{16}$ (R1210), are involved in glutathione-methyl-fluorescein transport (Ryu et al., 2000). Similarly, deletion of amino acids 1–188 (Fernandez et al., 2002), the R412G change (Hulot et al., 2005), and an I1173F replacement (Keitel et al., 2003) in two Dubin-Johnson variants impaired leukotriene-C4 (LTC4) transport. In the rat protein, K325M ($TM_6$), D329N ($TM_6$), and R586L ($TM_{11}$) mutations significantly reduced both LTC4 and dinitrophenyl-glutathione (DNP-SG) transport (Ito et al., 2001b).

Studies using multiple substrates have demonstrated differential effects of mutations on the transport of different substrates, shedding light on an additional level of complexity. The W1254F mutation did not affect estradiol-17β-glucuronide (E2-17βG) transport, but almost completely erased LTC4 and methotrexate (MTX) transport by the protein (Ito et al., 2001a). As LTC4 was unable to block E2-17βG transport by this mutant, it was suggested by the researchers that this mutation was likely to abolish the binding of LTC4 to the mutant protein (Ito et al., 2001a), making W1254 the most C-terminus-proximal residue implicated in LTC4 transport. Moreover, several mutations in the rat Abcc2 were shown to affect substrate specificity (Ito et al., 2001b), with D329N and R588L being the most striking, as these mutations did not significantly modulate the transport of taurolitocholate-sulfate and E3040-glucuronide, whereas these changes inhibited the efflux of DNP-SG and LTC4. ABCC2/Abcc2 transports amphiphilic anionic conjugates as well as unconjugated organic anions. It was plausible to predict that amino acids with positively charged side chains may play a role in substrate binding and transport. Therefore, the two studies most extensively mapping amino acids defining transport as well as substrate specificity were focusing on basic amino acids in TM helices as well as in juxtamembrane domains (Ryu et al., 2000; Ito et al., 2001b). These studies established a role for K324, K483, R1210, and R1257 of the human protein (Ryu et al., 2000), as well as K325, D329, and R586 of the rat protein (Ryu et al., 2000; Ito et al., 2001b) in substrate transport. Additional proof confirming the validity of the original predictions is coming from mutations in Dubin-Johnson patients. Among these mutations recently compiled by Nies and Keppler (2007), of the five amino acids affected that are located outside the nucleotide-binding domains, four are basic (R100X, R393W, R1066X, and R1150H) and one is neutral (I1173F). R1150H is normally processed, but nonfunctional (Mor-Cohen et al., 2001). I1173F is improperly processed and degraded, but has been shown in membrane assays as nonfunctional (Keitel et al., 2003). For the R100X, R393W, and R1066X, *in vitro* expression and analysis needs to be carried out to explore if functional activity is affected by the mutation.

In sum, residues, many of them basic, playing a role in substrate transport are spread over the entire protein. For some Dubin-Johnson mutation,s further *in vitro* studies are granted to investigate if transport, protein folding, trafficking, and/or stability are affected by the mutation.

## Test systems

*In vivo* (mutant rats and knock-out mice) $TR^-$/GY and EHBR rats made it possible to characterize function well before cloning the protein. By the time cloning succeeded, it was known that ABCC2 plays a major role in the hepatic elimination of conjugates (Jansen et al., 1985; Mikami et al., 1986; Huber et al., 1987; Kuipers et al., 1988, 1989; Ishikawa et al., 1990; Kitamura et al., 1992; Nishida et al., 1992), as well as acidic drugs (Nishida et al., 1992; Elferink et al., 1995). The absence of Abcc2 from the canalicular membrane did not precipitate extensive hepatocellular damage, as Abcc3 upregulation reduced the accumulation of toxic conjugates (Kuroda et al., 2004; Johnson et al., 2006; Oswald et al., 2006b). A more modest upregulation of Abcc3 was also observed in the kidneys of $TR^-$ rats (Johnson et al., 2006). Upregulation of Abcc4 in the liver and kidneys of $TR^-$ rats were also observed at the mRNA, as well as protein, level (Chen et al., 2005). This finding explains the increased serum bile-salt levels in $TR^-$ rats (Jansen et al., 1985) and an increased sinusoidal bile-salt efflux in EHBR rats (Akita et al., 2001b), as Abcc4 was shown to transport bile salts (Rius et al., 2003). However, upregulation of Abcc4 in $TR^-$ rats was not confirmed by another group (Johnson et al., 2006). This apparent controversy has not been resolved yet. Interestingly, no compensatory upregulation of Abcc3 or Abcc4 was found in the intestine (Johnson et al., 2006). Recently, ABCC2 knock-out mice were generated (Chu et al., 2006; Vlaming et al., 2006). Studies on Abcc2 null mice have not clearly confirmed the compensatory, 6-fold upregulation of Abcc3 in the livers of $TR^-$ rats (Johnson et al., 2006). Only a 2-fold upregulation was observed in one study (Vlaming et al., 2006) and no upregulation in the other one (Chu et al., 2006). This latter observation conflicts with another study that found a modest (57%), but statistically significant, increase in hepatic Abcc3 in the same mouse strain (Nezasa et al., 2006). The two studies (Chu et al., 2006; Vlaming et al., 2006) differ in their conclusion

on hepatic Abcc4 expression, too, as one study did not detect upregulation in the liver (Vlaming et al., 2006), while the other observed a 6-fold increase (Chu et al., 2006). A 2-fold increase in the kidney was observed by both studies. One group (Vlaming et al., 2006) used mice with a mixed (50% 129/Ola, 50% FVB) background, and the other study used mice with a C57Bl/6 background (Chu et al., 2006). It has been noted that genetic background significantly affects Abcc3 expression in radixin-deficient mice (Fukumoto et al., 2007), as well as Abcc4 induction in the liver (Vlaming et al., 2006).

To gain further insights into compensatory functions of transporters, mice null for multiple transporters, such as Mdr1a/b$^{-/-}$, Abcc2$^{-/-}$ (Vlaming et al., 2006), Abcc2$^{-/-}$, Abcc3$^{-/-}$ (van de Wetering et al., 2007), and Abcc2$^{-/-}$, Abcg2$^{-/-}$ (Vlaming et al., 2009), were generated by crossing the respective knock-out mice. Studies characterizing mutations are discussed below.

Primary cell cultures, as well as immortalized and cancer cell lines, are important tools in drug research, as they are thought to have the full complement of transporters as well as metabolic enzymes characteristic of the respective barrier. Primary cultures usually form tight monolayers that allow for studying vectorial contribution of transporters to transcellular permeability. Primary hepatocytes dedifferentiate upon culturing under standard conditions and do not readily form monolayers. If rat hepatocytes are isolated as couplets, they retain bile canaliculi between them (Boyer and Meier, 1990) and have been shown to accumulate BCECF an ABCC2 substrate in the bile (Takeguchi et al., 1993). Several studies have been published to demonstrate transporter function in this experimental system (Maglova et al., 1995; Hayes et al., 1999; Mills et al., 1999; Crocenzi et al., 2001; Beuers et al., 2003; Crocenzi et al., 2003, 2005; Perez et al., 2006). Nevertheless, careful validation for hepatic drug transport has not been done in hepatocyte couplets. Small hepatocytes are hepatic progenitor cells that proliferate and form sharply delineated colonies upon extended culturing on collagen-coated surfaces (Mitaka et al., 1999; Sidler Pfandler et al., 2004). The cultures develop polarized transporter expression with the functional canalicular localization of Abcc2 (Sidler Pfandler et al., 2004; Sasaki et al., 2008). Primary hepatocytes also form canalicular networks when cultured between two layers of gelled collagen (Ryan et al., 1993; LeCluyse et al., 1994). This configuration, termed sandwich culture, has been applied for hepatocytes from many species, such as the rat (LeCluyse et al., 1994), humans (Ryan et al., 1993; Kono et al., 1997), mouse (Contreras and Talamantes, 1999), monkey, and dog (Lau et al., 2002). This experimental set-up has been extensively characterized for transporter studies (Lau et al., 2002; Ghibellini et al., 2007; Swift et al., 2009; Brower et al., this issue). The caveat of primary systems is specificity. As no ABCC2-specific inhibitors are available, knock-down techniques (Tian et al., 2004) or correlation studies with transporter-specific systems, such as vesicles overexpressing ABCC2/Abcc2 (Heredi-Szabo et al., 2009), are the present options. Availability and price of human hepatocytes are also limiting the utilization of this experimental system. Transfected cell models can overcome some of these limitations. Single transfectants expressing ABCC2/Abcc2, however, have limited use, as they can be only applied to a subset of substrate with modest passive permeability (Cui et al., 1999; Evers et al., 2000; Huisman et al., 2005; Marchetti et al., 2007). To overcome the limitation, low passive permeability poses a series of double transfectants coexpressing an uptake transporter in the basolateral membrane and ABCC2 in the apical membrane have been developed on the MDCKII (Cui et al., 2001; Sasaki et al., 2002, 2004; Keppler, 2005; Yamada et al., 2007; Ishiguro et al., 2008) and LLC-PK1 (Spears et al., 2005) background. These reagents are primarily used as hepatocyte models, as the rat Abcc2 (Sasaki et al., 2004; Liu et al., 2006) or human ABCC2 (Cui et al., 2001; Keppler, 2005; Spears et al., 2005; Yamada et al., 2007; Ishiguro et al., 2008) is coexpressed with either OATP1B1 or OTP1B3 uptake transporters characteristic of hepatocytes. The OATP2B1/ABCC2 double transfectant also exists, and these transporters are coexpressed in many other tissues and cell types and, therefore, can be considered as models of cell types other than hepatocytes, as well. Nevertheless, the MDCKII-OTP2B1/ABCC2 has only been evaluated as a hepatocyte model (Kitamura et al., 2008). As the substrate specificity of ABCC2 only partially overlaps with the substrate specificity of any of the three OATPs, a quadruple transfectant expressing OATP1B1, OATP1B3, and OATP2B1 on the basolateral membrane and ABCC2 on the apical membrane has been generated and characterized (Kopplow et al., 2005). Despite the availability of the most relevant double transfectants and the quadruple transfectant, data on only a limited set of compounds have been published. The relatively complex kinetics (Bartholome et al., 2007), and the lack of metabolic capability of the parental MDCKII and LLC-PK1 cells, is a limitation, too.

None of the above limitations pose a problem in the membrane assay systems. Two assay types utilize membranes overexpressing ABCC2: the ATPase assay and the vesicular transport assay (reviewed in Glavinas et al., 2008). The ATPase activity of ABC transporters is coupled with a substrate transport (reviewed in Ambudkar et al., 2003). The vesicular transport (VT) measures ATP-dependent transport of substrates into inside-out vesicles. The VT is generally considered more informative, as it allows the determination of the $K_m$ value for the transport of the respective substrate. However, for high passive permeability substrates, the ATPase assay is the

only *in vitro* assay that indicates the transport nature of the interaction (reviewed in Glavinas et al., 2008).

Membranes can be prepared from tissues, transfected cell lines, or cell lines infected with viruses harboring the transporter, cDNA. A number of studies have been carried out from using rat canalicular membrane vesicle (CMV) preparations (reviewed in Nies and Keppler, 2007). This assay system is particularly useful for Abcc2, as CMVs from TR$^-$ or EHBR rats could be used as controls. The other popular test system is the insect cell-baculovirus system. The transgene expression makes membranes prepared from infected insect cells ideal for ATPase studies (Sarkadi et al., 1992). Only two mammalian cell lines are known that express the transporter at a level suitable for ATPase assay. One overexpresses hamster Abcb1 (Callaghan et al., 1997) and the other overexpresses human ABCG2 (Glavinas et al., 2007).

Transporters expressed in insect cells are underglycosylated. However, underglycosylation does not seem to affect transporter function (Bakos et al., 1996; Gao et al., 1996; Pal et al., 2007). The lipid environment also differs between the insect and mammalian membranes, with the about 4–10-fold lower cholesterol content of insect cells being the most significant factor (Gimpl et al., 1995; Pal et al., 2007). The difference in cholesterol content does not make the insect cell system irrelevant, as cholesterol loading of insect membranes does not change the $K_m$ values for the substrates (Pal et al., 2007; Telbisz et al., 2007; Kis et al., 2009a, 2009b). This is in line with data on LTC4 transport mediated by human ABCC2, as the respective $K_m$ values are 1 μM in MDCKII membranes (Cui et al., 1999), 0.694 μM in Sf9 membranes (Heredi-Szabo et al., 2008), and 0.560 μM in reconstituted proteoliposomes (Hagmann et al., 2002). In contrast, cholesterol loading significantly increases transport rates of ABCB1 [also known as P-glycoprotein (P-gp) or multidrug-resistant protein 1 (MDR1)] (Kimura et al., 2007), ABCG2 (Pal et al., 2007; Telbisz et al., 2007), and ABCB11 (bile-salt export pump; BSEP) (Kis et al., 2009a). MDR-ABC transporters ABCB1 [also known as P-glycoprotein (P-gp) or multidrug-resistant protein 1 (MDR1)] and ABCG2 are known to localize in cholesterol-rich membrane domains (Bacso et al., 2004; Storch et al., 2007). ABCC2/Abcc2 was also shown to localize to cholesterol-rich membrane microdomains in hepatocytes (Tietz et al., 2005), and cholesterol extraction reduced the transport rate of Abcc2 in the canalicular membranes of mice (Paulusma et al., 2009). Along with increased transport rate, cholesterol loading potentiates the ATPase activity of ABCB1 (Kimura et al., 2007), ABCG2 (Pal et al., 2007; Telbisz et al., 2007), and ABCB11 (Kis et al., 2009b). The ATPase activity of ABCC2 has been studied in detail in membrane vesicles, but the effect of membrane cholesterol has not been determined (Bakos et al., 2000).

## Physiological substrates

ABCC2/Abcc2 transports a number of endogenous compounds conjugated to glucuronate, glutathione, and sulfate (Keppler and Konig, 2000; Deeley et al., 2006), as summarized in Table 2. Several techniques are used to identify physiological compounds accepted by ABCC2/Abcc2 as a substrate, including *in vivo* hepatobiliary elimination studies with mutant, Abcc2-deficient rat and mice (i.e., TR$^-$, EHBR, and Abcc2$^{-/-}$ mice) (Elferink et al., 1989; Hosokawa et al., 1992; Vlaming et al., 2006) studies, using membrane vesicles from normal and Abcc2-deficient rat liver (Jedlitschky et al., 1997) or from ABCC2/Abcc2-transfected cells (Kamisako et al., 1999). The main physiological differences observed between mutant strains and wild-type counterparts are decreased bile flow, increased plasma levels of bilirubin glucuronides, a strong reduction in the biliary secretion of reduced glutathione (GSH) and that of a range of glutathione, sulfate conjugates, and unconjugated compounds (Elferink et al., 1995; Akita et al., 2001b; Konig et al., 2003; Vlaming et al., 2006).

Since even ABCC2-deficient DJS patients and Abcc2-deficient mutants do not have severe liver damage, the loss or decreased function of ABCC2/Abcc2 itself is not the major cause of cholestasis in acquired hepatobiliary diseases of naïve individuals. In a 30-year follow-up of 10 patients with DJS, no death from cholestatic liver disease occurred, supporting the benign nature of DJS, despite acute episodes of anorexia and abdominal pain (Machida et al., 2005). However, several lines of evidence strongly suggest that sufferers of ABCC2/Abcc2 dysfunction are more susceptible to adverse drug effects causing cholestatic liver diseases. Identification of physiological substrates of ABCC2/Abcc2 helped to elucidate the contribution of ABCC2/Abcc2 in the maintenance of hepatobiliary homeostasis.

Excretion of bilirubin glucuronides is the rate-limiting step of bilirubin transport from the blood to bile (Arias et al., 1961; Raymond and Galambos, 1971). In DJS patients and Abcc2-deficient strains, bilirubin glucuronides accumulate in the blood and liver and are eliminated largely by renal excretion (Rosenthal et al., 1981; Hosokawa et al., 1992; Elferink et al., 1995; Machida et al., 2005). In TR$^-$/EHBR rats, the levels of bilirubin conjugates in plasma were 50–100 times higher than those in Wistar or Sprague-Dawley (SD) rats, respectively. In adult mutants, total serum bilirubin amounted to 33 μM, of which 81.8% was bisglucuronosylbilirubin (BGB) and 12.1% monoglucuronosylbilirubin (MGB), while in wild-type rats, unconjugated bilirubin (UCB) serum concentration, representing 90% of total pigments, reached only 0.3 μM (Jansen et al., 1985). Obstruction, adverse drug reaction, or inflammation-induced cholestasis has been associated with the endocytic retrieval of ABCC2/Abcc2 and concomitant impaired transport of bilirubin

conjugates and other ABCC2/Abcc2 substrates (Tanaka et al., 2002; Milkiewicz et al., 2003; Kojima et al., 2008). Two hours of biliary obstruction in rats caused a 44% drop in serum UCB concentration and a concomitant 10- and 30-fold increase of MGB and BGB level, respectively (Mesa et al., 1997). Similarly, rapid, significant increases in serum bilirubin-glucuronide concentrations were reported in cholestatic patients, in good correlation with impaired ABCC2 expression and function (Zollner et al., 2001; Milkiewicz et al., 2003; Yamada et al., 2005). In contrast, in Abcc2$^{-/-}$ mice, total and conjugated bilirubin serum levels increased only by 5- and 3-fold relative to wild type, respectively (Chu et al., 2006). Meanwhile, the biliary output of bilirubin, and its conjugates in Abcc2-deficient rats and mice, was only decreased to approximately 40 and 55% of normal (Jedlitschky et al., 1997; Vlaming et al., 2006). The major bilirubin conjugate in the bile of mutant rats is BGB, whereas in wild-type rat bile, MGB and BGB are present in almost equal proportions (Jedlitschky et al., 1997). The high level of bilirubin glucuronides in the bile of mutants indicates a low-affinity secretion of these conjugates through the canalicular membrane. A possible candidate for bilirubin-conjugate transport into the bile is ABCB1, as it transports other ABCC2 substrates, such as E2-17bG (Huang et al., 1998).

Kinetic parameters of MGB/BGB transport by ABCC2/Abcc2 were determined *in vitro* by VT assays. CMV from rat hepatocytes transported MGB and BGB at 8.9 and 8.5 pmol/min/mg protein, whereas CMV from liver lacking active Abcc2 did not transport the conjugates (Jedlitschky et al., 1997). In experiments using membrane vesicles from human embryonic kidney (HEK) cells expressing recombinant human or rat ABCC2/Abcc2, adenosine triphosphate (ATP)-dependent transport of MGB and BGB by human ABCC2 were 183 and 104 pmol/mg protein/min, respectively. $K_m$ values, regarding MGB and BGB transport, were 0.7 and 0.9 μM for ABCC2 and 0.8 and 0.5 μM for Abcc2, respectively (Kamisako et al., 1999).

In primary rat hepatocyte sandwich cultures, the canalicular transport rate of bilirubin conjugates was 8 pmol/$10^6$ cells/30 min; MGB and BGB were present in the canalicular space at almost equal concentrations. Inhibition of canalicular transport of bilirubin conjugates by cholestatic drugs increased the MGB:BGB ratio (Lengyel et al., 2008).

Conjugate transport by ABCC3/Abcc3 may serve as an overflow mechanism across the basolateral membrane when the canalicular secretion of ABCC2/Abcc2 substrates is impaired (Hirohashi et al., 1998; Teng and Piquette-Miller, 2007). This is substantiated by the elevated plasma concentration and renal clearance of bilirubin conjugates in Abcc2 mutants, DJS patients, and cholestatic rats. A study on bile-duct–ligated (BDL) GY/TR$^-$ and EHBR mutant rats highlighted that Abcc3, normally expressed at low levels, is upregulated when the canalicular secretion of conjugates is impaired (Hirohashi et al., 1999). In the liver, the mRNA expression of Abcc2 decreased by 59, 86, and 82%, and its protein expression decreased by 25, 74, and 93%, compared to sham-operated animals after 24, 72, and 120 hours of BDL, respectively. In contrast, treatment increased the liver expression of Abcc3 mRNA by 138, 2,137, and 3,295%, and its protein expression by 560, 634, and 612% after 24, 72, and 120 hours of BDL, respectively (Tanaka et al., 2002). This upregulation of ABCC3 was also observed in studies on the human liver. Livers from patients with DJS and patients with primary biliary cirrhosis exhibited high ABCC3 expression in the basolateral hepatocyte membrane, compared to apparently normal livers. Unlike in Abcc2 mutant rats, data on induction of Abcc3 in Abcc2$^{-/-}$ mice are controversial. A plausible explanation for this discrepancy might be that under normal conditions, the level of Abcc3 in mouse hepatocytes is much higher than levels in rats and humans; thus, mice have a constitutive mechanism to compensate for the impaired Abcc2 activity (Belinsky et al., 2005).

Several papers reported a role of ABCC2/Abcc2 in the transport of steroid glucuronides, especially that of E2-17βG. This substrate is commonly used for standardizing ABCC2/Abcc2 expression and activity of molecular biology products and transgenic mutant strains. However, the mechanism of E2-17βG transport by ABCC2/Abcc2 is still much debated. In a recent article, Borst et al. (2006b) reviewed contradictory results obtained in different labs. Table 2 depicts some of the available kinetic parameters; the kinetics of E2-17βG transport by ABCC2/Abcc2 will be discussed later in more detail.

The hepatobiliary disposition of thyroxine (T4) was evaluated in TR$^-$ rats. Serum concentrations of T4 were approximately 50% higher in TR$^-$ rats than in Wistar. Total biliary clearance (Cl(bile)) was approximately 0.85 and 0.2 mL/h in Wistar and TR$^-$ rats, respectively, with virtually no T4-glucuronide excreted to the bile of TR$^-$ rats. Biliary clearance of unconjugated T4 was also lower in TR$^-$ rats (Lecureux et al., 2009). These findings indicate that T4-glucuronide is an Abcc2 substrate; still, other mechanisms seem more crucial for maintaining the T4 homeostasis.

Abbc2 is also involved in the biliary transport of GSH and oxidized glutathione (GSSG). Liver is the major site of GSH synthesis. In rat liver, approximately one half of the GSH is released into the plasma, and the remaining half is secreted into the bile, with biliary GSH concentrations reaching 8–10 mM (Ballatori et al., 2005). Bile salts and GSH are the major driving force for bile-salt–dependent and –independent bile flow, respectively (Ballatori and Truong, 1992). The mechanism of biliary secretion of GSH and GSSG is not completely defined; it is proposed to be partially mediated by Abcc2 (Suzuki

**Table 2.** Physiological substrates of ABCC2/Abcc2.

| Compound | System | *In vitro* kinetic parameters | Reference |
|---|---|---|---|
| Glucuronide conjugates | | | |
| Bilirubin bisglucuronosyl | HEK-MRP2; VT | $K_m$: 0.9 μM | Kamisako et al., 1999 |
| | HepG2; VT | Transport rate: 4.4 pmol/mg/min | Jedlitschky et al., 1997 |
| | HEK-rat Mrp2; VT | $K_m$: 0.5 μM | Kamisako et al., 1999 |
| | Rat CMV; VT | Transport rate: 8.5 pmol/mg/min | Jedlitschky et al., 1997 |
| Bilirubin monoglucuronosyl | HEK-MRP2; VT | $K_m$: 0.7 μM | Kamisako et al., 1999 |
| | HepG2; VT | Transport rate: 8.3 pmol/mg/min | Jedlitschky et al., 1997 |
| | HEK-rat Mrp2; VT | $K_m$: 0.8 μM | Kamisako et al., 1999 |
| | Rat CMV; VT | Transport rate: 8.9 pmol/mg/min | Jedlitschky et al., 1997 |
| E217βG | HEK293-MRP2; VT | $K_m$: 7.2 μM | Cui et al., 1999 |
| | Sf9-MRP2; VT | $K_{50}$: 120 μM | Zelcer et al., 2003 |
| | Sf9-MRP2; VT | $K_{50}$: 150 μM | Heredi-Szabo et al., 2009 |
| | Human CMV; VT | $K_m$: 364 μM | Shilling et al., 2006 |
| | HEK293- rat Mrp2; VT | $K_m$: 6.9 μM | Cui et al., 1999 |
| | Sf9-rat Mrp2; VT | $K_m$: 3.9 μM | Ito et al., 2001b |
| | Sf9-rat Mrp2; VT | $K_m$: 16 μM | Borst et al., 2006a |
| | Sf9-rat Mrp2; VT | $K_m$: 4.8 μM | Ninomiya et al., 2005 |
| | Sf9-rat Mrp2; VT | $K_m$: 61.5 μM | Heredi-Szabo et al., 2009 |
| | Rat CMV; VT | $K_m$: 465 μM | Shilling et al., 2006 |
| Thyroxine | In vivo WR/TR- | $Cl_{bile}$: 0.85/0.2 mL/h; no T4-G in bile | Lecureux et al., 2009 |
| GSH and GSH conjugates | | | |
| GSH | MDCK-MRP2; ME | Transport rate: 141 pmol/min/ mg prot | Paulusma et al., 1999 |
| | MDCKII-Mrp2; ME | $K_{m:}$ >2 mM | Lou et al., 2003 |
| | Rat CMV; VT | | Rebbeor et al., 2002 |
| | Rat Mrp2; *in vivo* biliary output | Male: 2.61 nmol/min/g liver wild type; female: 2.23 nmol/min/g liver wild type | Veggi et al., 2005 |
| | Rat; *in vivo* biliary output | Male: 0.22 nmol/min/g liver wild type; female: 0.18 nmol/min/g liver wild type | Veggi et al., 2005 |
| GSSG | Rat CMV; VT | $K_m$: 111 μM | Leier et al., 1996 |
| Cysteinyl leucotrienes | | | |
| $LTC_4$ | HEK293-MRP2; VT | $K_m$: 1 μM | Cui et al., 1999 |
| | HEK293-rat Mrp2; VT | $K_m$: 1.1 μM | Cui et al., 1999 |
| | Rat CMV; VT | $K_m$: 0.3 μM | Ishikawa et al., 1990 |
| | | $K_m$: 0.72 μM | Shilling et al., 2006 |
| $LTD_4$ | Rat CMV; VT | $K_m$: 1.5 μM | Ishikawa et al., 1990 |
| $LTE_4$ | Rat CMV; VT | $K_m$: > 10μM | Ishikawa et al., 1990 |
| N-acetylated $LTE_4$ | Rat CMV; VT | $K_m$: 5.2 μM | Ishikawa et al., 1990 |
| Sulfate conjugates | | | |
| Dehydroepiandrosterone sulfate | MDCKII-MRP2/OATP1B3 ME | n.a. | Cui et al., 2001 |
| Estron-3-sulfate | MDCKII-MRP2; VT MDCK-MRP2/ OATP1B1/1B3/2B1; ME | n.a. | Kopplow et al., 2005 |
| Taurolithoholate sulfate | Rat CMV; VT | $K_m$: 1.5 μM | Akita et al., 2001a |
| | Sf9-rat Mrp2;VT | $K_m$: 3.9 μM | Akita et al., 2001a |
| Taurochenodeoxycholate sulfate | Rat CMV; VT | $K_m$: 8.8 μM | Akita et al., 2001a |

CMV, canalicular membrane vesicles; ME, monolayer efflux assay (vectorial transport assay); n.a., no data available; $TR^-$, Groningen Yellow transport deficient rat; VT, vesicular transport; WR, Wistar rat.

and Sugiyama, 1998; Konig et al., 1999; Paulusma et al., 1999). The first indication that Abcc2 is involved in GSH transport came from studies with $TR^-$ and EHBR mutant rats. Mutant rats were unable to secrete GSH and glutathione *S*-conjugates into the bile, and a dramatic decrease in biliary GSH secretion was described in Abcc2$^{-/-}$ mice, too (Jansen et al., 1985; Takikawa et al., 1991; Vlaming et al., 2006). Bile flow in mutants was 50% lower, compared to wild-type counterparts (Jansen et al., 1985; Paulusma et al., 1999; Akita et al., 2001a; Vlaming et al., 2006), and intracellular GSH levels were high, too. In Abcc2-deficient rats and mice, hepatic GSH levels increased by approximately 2-fold (Ballatori et al., 1995; Chu et al., 2006). Studies with rat-liver CMV provided

the first direct evidence for GSH transport by Abcc2 (Rebbeor et al., 2002). GSH is transported with low catalytic efficiency and relatively high $K_m$ and low $V_{max}$ values, leading to a low $V_{max}/K_m$ ratio. The high $K_m$ values are not unexpected, as GSH is present at high concentrations within cells (1–10 mM). Uptake experiments with CMV have suggested that GSH is a substrate for human and rat ABCC2/Abcc2, with an apparent $K_m$ in the millimolar range (Paulusma et al., 1999). Other studies have also reported the correlation of Abcc2 expression levels and GSH transport rates. BDL led to a slow decrease in GSH level in the bile that was restored after the BDL had been reversed; the changes were in good correlation with Abcc2 protein expression (Paulusma et al., 2000). The endocytic retrieval of ABCC2/Abcc2 in livers exposed to cholestatic drugs, as well short durations of warm or cold ischemia, was a major cause of decrease in biliary GSH elimination and bile flow (Kojima et al., 2008; Rost et al., 2008; Ban et al., 2009). On the contrary, the herbicide, 2,4,5-trichlorophenoxyacetic acid, induced Abcc2 gene expression in mouse liver and increased biliary GSH excretion (Wielandt et al., 1999). Likewise, the choleretic effect of genipin was connected with increased Abcc2 protein expression in the CMVs and a marked increase in Abcc2 density in the canalicular membrane and microvilli. It also increased biliary secretion of bilirubin conjugates and GSH by 513 and 336%, respectively, but did not increase bile-acid secretion. The ATP-dependent uptake of E2-17bG, LTC4, and taurolithocholate-3-sulfate was also significantly stimulated (by 265, 161, and 266%, respectively) in the liver CMV of genipin treated rats. These effects were not observed in Abcc2-deficient rats (Shoda et al., 2004). By exporting both GSH and GSSG, ABCC/Abcc transporters are involved in the regulation of the cellular thiol-redox status through the control of the GSSG:GSH ratio and in the defense against oxidative stress under conditions of enhanced GSSG formation (Keppler and Konig, 2000; Veggi et al., 2005).

In a recent article, Ballatori and coworkers reviewed transporters involved in GSH homeostasis in normal and pathologic conditions (Ballatori et al., 2009). Several mechanisms were proposed for the transport of GSH by ABCC/Abcc proteins. One mechanism could be that GSH itself is a substrate of Abcc2, yet a relatively poor substrate. In contrast, GSSG is transported by Abcc1 with a much higher $V_{max}$ and a $K_m$ of 100 μM (Paulusma et al., 1999; Rebbeor et al., 2002). Cotransport of GSH with another substrate is a further possible mechanism of GSH transport. In the presence of GSH-dependent substrates or modulators of Abcc1, the $K_m$ for GSH decreases to approximately the same range as GSSG (Deeley et al., 2006). Transport of compounds, such as vinblastine, vincristine, and etoposide, by Abcc1 was GSH dependent (Cui et al., 1999). Similarly, vinblastine and GSH are cotransported by Abcc2 (Evers et al., 2000) and GSH appears to stimulate the ATPase activity of ABCC2 (Bakos et al., 2000). A third possibility is that the transport of certain substrates is stimulated by, or is dependent on, GSH, but GSH itself is not transported (Leslie et al., 2001). A fourth possible mechanism is that GSH transport is stimulated by drugs, such as verapamil and bioflavins, by reducing the $K_m$ value for GSH by 10-fold; however, these compounds themselves are not transported by Abcc transporters (Leslie et al., 2001; Lou et al., 2003).

The endogenous glutathione *S*-conjugate, LTC4, due to its high affinity, is a widely used model substrate for ABCC1/Abcc1. ABCC2/Abcc2 also transports LTC4 and its metabolites; however, the affinity of ABCC2 for LTC4 is 10-fold lower, compared to ABCC1 (Cui et al., 1999). Biliary efflux studies with Abcc2-deficient rats clearly demonstrated that these mutants cannot excrete LTC4 and its metabolites into the bile. Consequently, conjugates accumulate within the hepatocytes and are, subsequently, pumped across the basolateral membrane into the blood, probably by Abcc1, and, finally, LTC4 metabolites are excreted to the urine (Jedlitschky et al., 1997, Konig et al., 1999, Suzuki and Sugiyama, 1998). First evidence on ATP-dependent transport of LTC4 came from studies on CMV and SMV isolated from rat liver. LTD4, LTE4, N-acetyl-LTE4, and omega-carboxy-N-acetyl-LTE4 were also transported in an ATP-dependent manner. Apparent $K_m$ values of the transport system for LTC4, LTD4, and N-acetyl-LTE4 were 0.25, 1.5, and 5.2 μM, respectively (Ishikawa et al., 1990). Direct evidence of ATP-dependent LTC4 transport by ABCC2 was provided by studies using cells expressing human ABCC2 (Madon et al., 1997). Vesicular uptake experiments, using membrane vesicles prepared from transiently transfected HEK-ABCC2 cells, proved that several structurally and biologically distinct classes of endogenous and exogenous GSH-conjugated catechol metabolites can inhibit both ABCC1- and ABCC2-mediated LTC4 transport (Slot et al., 2008). In rat CMV, LTC4 transport was inhibited by MGB and BGB, with $IC_{50}$ values of 0.12 and 0.10 μM, respectively (Jedlitschky et al., 1997), and, in contrast, LTC4 inhibited MGB transport in membrane vesicles originating from human ABCC2-transfected cells, with $IC_{50}$ values of 2.3 μM (Kamisako et al., 1999).

Although ABCC2/Abcc2 transports glucuronide conjugates more efficiently than sulfates, several endogenous sulfates, such as bile-salt sulfates, are among its substrates. Biliary excretion of certain bile acids by ABCC2/Abcc2 has been suggested; however, ABCB11/Abcb11 represents the major bile-salt transporter across the canalicular membrane of hepatocytes (Akita et al., 2001a). Inconsistent results have been published concerning bile-acid transport by ABCC2. Unlike ABCB11/Abcb11, which transports a broad range of nonsulfated

bile salts, ABCC2/Abcc2 transports only sulfated bile salts, such as taurochenodeoxycholate-3-sulfate (TCDC-S) and taurolithocholate-3-sulfate (TLC-S), but not monoanionic bile salts, such as taurocholate (Gerk and Vore, 2002). Due to its substrate specificity, ABCC2/Abcc2 plays a central role in detoxification by secreting metabolites into the bile (Liu et al., 2003). In contrast, active, concentration-dependent, saturable glycocholate uptake by ABCC2 was reported, with 100-pmol/mg membrane protein/min $V_{max}$ and 150-μM $K_m$ values from using human ABCC2-expressing Sf9 membrane vesicles (Bodo et al., 2003). ATP-dependent uptake of TCDC-S and TLC-S was observed in CMV isolated from SD rats, with a $K_m$ value of 8.8 and 1.5 μM, respectively, but no transport was observed in CMV of EHBR rats. Experiments using CMV from Abcc2-expressing *Spodoptera frugiperda 9 insect ovarian* (Sf9) cells confirmed the role of Abcc2 in the transport of TLC-S, with 3.9-μM $K_m$ and 1,486-pmol/min/mg protein $V_{max}$ values (Akita et al., 2001a).

It is still unclear whether estrone-3-sulfate and dehydroepiandrosterone sulfate are substrates of ABCC2 or not. Transcellular transport studies, using double-transfected MDCKII cell monolayers expressing OATP1B1 on the basal and ABCC2 on the apical membranes, revealed that estrone-3-sulfate and dehydroepiandrosterone sulfate are transported by OATP1B1, but not by ABCC2 (Sasaki et al., 2002). On the contrary, vectorial transport of estrone 3-sulfate was detected in all three double-transfected MDCKII cells (ABCC2/OATP1B1, ABCC2/OATP1B3, and ABCC2/OATP2B1), in ABCC2/OATP1B1/1B3/2B1 quadruple-transfected MDCKII cells, and of dehydroepiandrosterone sulfate in double-transfected ABCC2/OATP1B3-expressing cells, suggesting that estrone-3-sulfate and dehydroepiandrosterone sulfate is not only a substrate for OATP1B1, OATP1B3, and OATP2B1, but also for ABCC2. ATP-dependent uptake, using CMV prepared from single ABCC2-transfected cells, provided direct evidence that estrone-3-sulfate is a substrate for ABCC2 (Cui et al., 2001; Kopplow et al., 2005).

## Xenobiotic substrates

The number of compounds interacting with ABCC2 is large. More than 100 small-molecule inhibitors have been reported (Zhang, 2009). The interacting compounds studied in the most detail can be classified as drugs, nutraceuticals, toxicants, and fluorescent-probe substrates, as well as their conjugates. The broad array of chemicals reflects the importance of ABCC2/Abcc2 in protecting the tissues from potentially toxic xenobiotics, as well as its role in modulating the ADME properties of essential nutrients. Compounds that were experimentally shown as modulators (Table 3) and/or substrates (Table 4) have been compiled. Only data obtained by experimental systems that were ABCC2/Abcc2 specific are shown.

The interacting compounds are, structurally, very diverse. Majority of them are heterocyclic, but homocyclic compounds, as well as complexes, can also be found among them (Table 3 and 4). Various *in silico* methods have been used to determine molecular characteristics critical for interaction with ABCC2/Abcc2 (reviewed in Xing et al., 2009). Lipophilicity/ClogP (Han et al., 2001; Hirono et al., 2005; Ng et al., 2005; Pedersen et al., 2008; Zhang et al., 2009), hydrophobic/aromatic functions (Hirono et al., 2005; Pedersen et al., 2008; Zhang et al., 2009), negative charge (Hirono et al., 2005; Ng et al., 2005; Pedersen et al., 2008), H-bond donor feature (Hirono et al., 2005; Zhang et al., 2009), and greater than average molecular weight (Ng et al., 2005; Pedersen et al., 2008) were the dominant properties.

Three studies have attempted to build a pharmacophore model. All studies identified hydrophobic/aromatic and H-bond acceptor/anionic functions as crucial. However, the number of different pharmacophores varies. One study identified two hydrophobic/aromatic and one H-bond acceptor/anionic function (Ng et al., 2005). In contrast, another study defined one hydrophobic/aromatic and two H-bond acceptor/anionic functions (Zhang et al., 2009), while a third suggested a model with two hydrophobic/aromatic and two H-bond acceptor/anionic functions (Hirono et al., 2005). Discrepancy exists between the distance requirements of the two H-bond acceptor functions, too (Hirono et al., 2005; Zhang et al., 2009). The lack of correlation might stem from the different set of compounds used in the studies, as well as from the differences in the methods applied. In addition, two of the groups studied the rat Abcc2 protein (Hirono et al., 2005; Ng et al., 2005) and the third group used data generated on multiple species (Zhang et al., 2009).

Pedersen et al. (2008) tested a set of 191 structurally diverse compounds in Sf9-ABCC2 membranes to monitor the inhibition of estradiol-3β-glucuronide (E2-17βG) transport. In addition to determining features discriminating interactors and noninteractors, they also analyzed characteristics of transport inhibitors, transport stimulators, and known substrates. The latter group was, on average, less lipophilic than other inhibitors and also had a higher molecular weight and a larger polar surface area. Stimulators mainly differed from noninteracting compounds by a greater negative charge, larger number of functional groups involved in hydrogen bonding, lower lipophilicity, and smaller size. Interestingly, all stimulators had at least one negative charge at physiological pH. Intriguingly, the charge characteristics of stimulators were similar to those of substrates, suggesting that the stimulating binding site(s) may be similar to the transport

**Table 3.** Modulators of ABCC2/Abcc2.[a]

| Compound | System | Substrate | Computed values/kinetic parameters | References |
|---|---|---|---|---|
| Endogenous compounds | | | | |
| GC | Sf9-ABCC2; VT | [$^3$H]$E_2$17βG 1 μM | 180% of control at 100 μM of M | Bodo et al., 2003 |
| GCDC | Sf9-ABCC2; VT | [$^3$H]$E_2$17βG 1 μM | ~240% of control at 100 μM of M | Bodo et al., 2003 |
| LTC4 | Sf9-ABCC2; VT | CDCF 5 μM | $K_i$: 1.8 ± 0.3 μM | Heredi-Szabo et al., 2008 |
| TCDC | Sf9-ABCC2; VT | [$^3$H]$E_2$17βG 1 μM | ~210% of control at 100 μM of M | Bodo et al., 2003 |
| TDC | Sf9-ABCC2; VT | [$^3$H]$E_2$17βG 1 μM | 150% of control at 100 μM of M | Bodo et al., 2003 |
| Drugs and drug conjugates | | | | |
| Benzbromarone | Sf9-ABCC2; VT | [$^3$H]LTC4 50 nM | $K_i$: 24 ± 6 μM | Heredi-Szabo et al., 2008 |
| | Sf9-ABCC2; VT | CDCF 5 μM | $K_i$: 4.4 ± 1.3 μM | Heredi-Szabo et al., 2009 |
| | Sf9-ABCC2; VT | [$^3$H]$E_2$17βG 1 μM | 430% of control at 10 μM of M | Heredi-Szabo et al., 2009 |
| | Sf9-rAbcc2; VT | [$^3$H]$E_2$17βG 1 μM | 155% of control 11 μM of M | Heredi-Szabo et al., 2009 |
| | MDCKII-ABCC2; GSH efflux | GSH | 450% stimulation of apical GSH transport at 5 μM of M | Evers et al., 2000 |
| Bromosulphthalein | MDCKII-ABCC2; ME | [$^3$H]CDNB 2 μM | 84 ± 5% of control at 100 μM BS | Evers et al., 1998 |
| Celecoxib | MDCKII-ABCC2; VT | [$^3$H]MTX 0.5 μM | $IC_{50}$: 100 ± 2 μM | El-Sheikh et al., 2007 |
| Cyclosporin A | Rat CMV; VT | [$^3$H]DNP-SG 1 μM | $K_i$: 1.96 μM | Horikawa, 2002 |
| | LLC-PK1-ABCC2; VT | [$^3$H]LTC4 1.37 nM | $K_i$: 4.7 μM | Chen et al., 1999 |
| | MDCKII-ABCC2; DE | Calcein AM 0.5 μM | $IC_{50}$: 13.6 ± 1.9 μM | Leyers, 2008 |
| Diclofenac | MDCKII-ABCC2; VT | [$^3$H]MTX 0.5 μM | $IC_{50}$: 97 ± 1 μM | El-Sheikh et al., 2007 |
| Etodolac | MDCKII-ABCC2; VT | [$^3$H]MTX 0.5 μM | $IC_{50}$: 480 ± 2 μM | El-Sheikh et al., 2007 |
| Furosemide | Sf9-ABCC2; VT | [$^3$H]$E_2$17βG 13 μM | Up to 150% of control at 50–500 μM of M | Bodo et al., 2003 |
| GS-*N*-Me-α-MeDA | HEK293-ABCC2; VT | [$^3$H]$E_2$17βG 0.1–30 μM | $IC_{50}$: 145 ± 46 μM | Slot et al., 2008 |
| Ibuprofen | MDCKII-ABCC2; VT | [$^3$H]MTX 0.5 μM | $IC_{50}$: 930 ± 20 μM | El-Sheikh et al., 2007 |
| Indomethacin | MDCKII-ABCC2; VT | [$^3$H]MTX 0.5 μM | $IC_{50}$: 0.06 ± 0.01 μM high affinity; 46 ± 1 μM low affinity; | El-Sheikh et al., 2007 |
| | Sf9-ABCC2; VT | [$^3$H]LTC4 50 nM | $K_i$: 152 ± 89 μM | Heredi-Szabo et al., 2008 |
| | Sf9-ABCC2; VT | CDCF 5 μM | $K_i$: 71 ± 24 μM | Heredi-Szabo et al., 2008 |
| | MDCKII-ABCC2; DE | Calcein AM 0.5 μM | $IC_{50}$: 235 ± 73 μM | Leyers et al., 2008 |
| | MDCKII-ABCC2; ME | [$^3$H]CDNB 2 μM | 109 ± 4% of control at 100 μM of M | Evers et al., 1998 |
| | MDCKII-ABCC2; GSH efflux | GSH 1 μM | 250% stimulation of apical GSH transport at 100 μM of M | Evers et al., 2000 |
| | Sf9-ABCC2; VT | [$^3$H]$E_2$17βG 13 μM | 6–6.5-fold stimulation of control at 50–100 μM of M | Bodo et al., 2003 |
| | Sf9-ABCC2; VT | [$^3$H]$E_2$17βG 1 μM | 750% of control at 100 μM | Heredi-Szabo et al., 2009 |
| | Sf9-rAbcc2; VT | [$^3$H]$E_2$17βG 1 μM | 510% of control at 270 μM of M | Heredi-Szabo et al., 2009 |
| Irinotecan carboxylate form | Rat CMV; VT | [$^3$H]DNP-SG 1 μM | $K_i$: 96.6 ± 8.4 μM | Chu et al., 1997 |
| Irinotecan active metabolite: SN-38 carboxylate form | Rat CMV; VT | [$^3$H]DNP-SG 1 μM | $K_i$: 18.3 ± 3.6 μM | Chu et al., 1997 |
| Irinotecan active metabolite-Glu: SN-38-Glu carboxylate form | Rat CMV; VT | [$^3$H]DNP-SG 1 μM | $K_i$: 1.03 ± 0.05 μM | Chu et al., 1997 |
| Irinotecan active metabolite-Glu: SN-38-Glu lactone form | Rat CMV; VT | [$^3$H]DNP-SG 1 μM | $K_i$: 1.62 ± 0.05 μM | Chu et al., 1997 |
| Ketoprofen | MDCKII-ABCC2; VT | [$^3$H]MTX 0.5 μM | $IC_{50}$: 1.4 ± 0.1 μM high affinity; 470 ± 20 μM low affinity | El-Sheikh et al., 2007 |
| Naproxen | MDCKII-ABCC2; VT | [$^3$H]MTX 0.5 μM | $IC_{50}$: 609 ± 7 μM | El-Sheikh et al., 2007 |
| Phenylbutyzone | MDCKII-ABCC2; VT | [$^3$H]MTX 0.5 μM | $IC_{50}$: 605 ± 4 μM | El-Sheikh et al., 2007 |
| Piroxicam | MDCKII-ABCC2; VT | [$^3$H]MTX 0.5 μM | $IC_{50}$: 257 ± 2 μM | El-Sheikh et al., 2007 |

Table 3. continued on next page

**Table 3.** Continued.

| Compound | System | Substrate | Computed values/kinetic parameters | References |
|---|---|---|---|---|
| Probenecid | Sf9-ABCC2; VT | [3H]LTC4 50 nM | $K_i$: 2,300 μM | Heredi-Szabo et al., 2008 |
| | Sf9-ABCC2; VT | CDCF 5 μM | $K_i$: 580 ± 45 μM | Heredi-Szabo et al., 2008 |
| | Primary hepatocytes; DE | CDCF | ~3-fold increase in retention in the presence of 2.5–20 mM of M | Payen et al., 2000 |
| | Rat CMV; VT | [$^3$H]DNP-SG 1 μM | $K_i$: 44.6 μM | Horikawa et al., 2002 |
| | MDCKII-ABCC2; ME | [$^3$H]CDNB 2 μM | 83 ± 4% of control at 10 mM of M | Evers et al., 1998 |
| | Sf9-ABCC2; VT | [$^3$H]$E_2$17βG 13 μM | Up to 150% of control At 50–500 μM of M | Bodo et al., 2003 |
| | Sf9-ABCC2; VT | [$^3$H]$E_2$17βG 0.5–1 μM | 265% of control at 330 μM of M | Heredi-Szabo et al., 2009 |
| | Sf9-rAbcc2; VT | [$^3$H]$E_2$17βG 0.5–1 μM | 280% of control at 1,100 μM of M | Heredi-Szabo et al., 2009 |
| Rifampicin | HEK-ABCC2; VT | [$^3$H]LTC4 | LTC4 transport 50% of control at 50 μM of M | Cui et al., 2001 |
| Rifampycin SV | HEK-ABCC2; VT | [$^3$H]LTC4 | LTC4 transports 38% of control at 50 μM of M | Cui et al., 2001 |
| Salycilate | MDCKII-ABCC2; VT | [$^3$H]MTX 0.5 μM | $IC_{50}$: 1,760 ± 30 μM | El-Sheikh et al., 2007 |
| Sulfasalazine | Sf9-ABCC2; VT | [$^3$H]LTC4 50 nM | $K_i$: 25 ± 15 μM | Heredi-Szabo et al., 2008 |
| | Sf9-ABCC2; VT | CDCF 5 μM | $K_i$: 16 ± 6 μM | Heredi-Szabo et al., 2008 |
| Sulfasalazine[a] | Sf9-ABCC2; VT | [$^3$H]$E_2$17βG 1 μM | 430% of control at 35 μM of M | Heredi-Szabo et al., 2009 |
| | Sf9-rAbcc2; VT | [$^3$H]$E_2$17βG 1 μM | 360% of control at 110 μM of M | Heredi-Szabo et al., 2009 |
| Sulfinpyrazone[a] | MDCKII-ABCC2; ME | [$^3$H]CDNB 2 μM | 130 ± 3% of control at 2 mM of M | Evers et al., 1998 |
| Sulindac | MDCKII-ABCC2; VT | [$^3$H]MTX 0.5 μM | $IC_{50}$: 38 ± 1 μM | El-Sheikh et al., 2007 |
| Tolmetin | MDCKII-ABCC2; VT | [$^3$H]MTX 0.5 μM | $IC_{50}$: 494 ± 5 μM | El-Sheikh et al., 2007 |
| Verapamil | MDCKII-ABCC2; DE | Calcein AM 0.5 μM | $IC_{50}$: > 200 μM | Leyers et al., 2008 |
| Inhibitors not in clinical use | | | | |
| MK571 | LLC-PK1-ABCC2; VT | [$^3$H]LTC4 1.37 nM | $K_i$: 13.1 μM | Chen et al., 1999 |
| | Sf9-ABCC2; VT | [$^3$H]LTC4 50 nM | $K_i$: 1.8 ± 0.5 μM | Heredi-Szabo et al., 2008 |
| | Sf9-ABCC2; VT | CDCF 5 μM | $K_i$: 4.1 ± 1.1 μM | Heredi-Szabo et al., 2008 |
| | MDCKII-ABCC2; DE | Calcein AM 0.5 μM | $IC_{50}$: 20.7 ± 7.3 μM | Leyers et al., 2008 |
| PAK-104P | LLC-PK1-ABCC2; VT | [$^3$H]LTC4 1.37 nM | $K_i$: 3.7 μM | Chen et al., 1999 |
| PSC833 | LLC-PK1-ABCC2; VT | [$^3$H]LTC4 1.37 nM | $K_i$: 28.9 μM | Chen et al., 1999 |
| Nutraceuticals and their conjugates | | | | |
| 2-GS-CA | HEK293-ABCC2; VT | [$^3$H]$E_2$17βG 0.1–30 μM | $IC_{50}$: 9.6 – 10.2 μM | Slot et al., 2008 |
| Baicalin | Rat CMV; VT | [$^3$H]DNP-SG 1 μM | $K_i$: 7.95 μM | Horikawa et al., 2002 |
| Genistein | Rat CMV; VT | [$^3$H]DNP-SG 1 μM | $K_i$: 16.1 μM | Horikawa et al., 2002 |
| Glycyrrhetic acid | Sf9-ABCC2; VT | [$^3$H]$E_2$17βG 0.1 μM | $IC_{50}$: 20.1 ± 3.1 μM | Yoshida et al., 2008 |
| Toxic compounds | | | | |
| Abietic acid | Sf9-ABCC2; VT | [$^3$H]$E_2$17βG 0.1 μM | $IC_{50}$: 51.4 ± 6.7 μM | Yoshida et al., 2008 |
| Fluorescent and other test compounds and their conjugants | | | | |
| CDCF | Sf9-ABCC2; VT | [$^3$H]LTC4 50 nM | $K_i$: 15 ± 10 μM | Heredi-Szabo et al., 2008 |
| 4-[5,6,7,8-tetrahydro-4-oxo-4H-[1]benzothieno[2,3-d][1,3]thiazin-2-ylamino] benzoic acid | MDCKII-ABCC2; DE | Calcein AM 0.5 μM | $IC_{50}$: 21.5 ± 5.9 μM | Leyers et al., 2008 |
| Sulfobromophthalein | Rat CMV; VT | [$^3$H]DNP-SG 1 μM | $K_i$: 2.18 μM | Horikawa et al., 2002 |
| Sulfobromophthalein-SG | Rat CMV; VT | [$^3$H]DNP-SG 1 μM | $K_i$: 0.046 μM | Horikawa et al., 2002 |

[a]Compounds that inhibit or potentiate ABCC2/Abcc2-mediated transport of known probe substrates.
CDCF, carboxydichlorofluorescein; CMV, canalicular membrane vesicles; DE, dye efflux assay; M, modulator concentration; ME, unidirectional monolayer assay transport to apical side; Sf9, *Spodptera frugiperda* 9; VT, vesicular transport assay.

binding site. However, the group of stimulators is not restricted to substrates (Zelcer et al., 2003; Borst et al., 2006b).

To accommodate the large, diverse set of interacting compounds, two different binding-site models have been proposed, such as one large binding site and multiple smaller binding sites (Borst et al., 2006b). Based on their data, Hirono et al. (2005) proposed a model with two electrostatically positive and two hydrophobic areas as part of the primary binding sites and two electrostatically

**Table 4.** Substrates of ABCC2/Abcc2.

| Compound | System | Computed values /*in vitro* kinetic parameters | References |
|---|---|---|---|
| Drugs and drug conjugates | | | |
| Belotecan | MDCKII/ABCC2; ME | $K_m$: 269.93 mM | Li et al., 2008 |
| Cisplatin | LLC-hABCC2; CT | RR: 7 | Kawabe et al., 1999 |
| | HEK-hABCC2; CT | RR: 10 | Cui et al., 1999 |
| Docetaxel | MDCKII-ABCC2/Neo; ME | ER: ABCC2: 8.2 ± 0.1; Neo: 1.3 ± 0.3 | Huisman et al., 2005 |
| Doxorubicin | HEK-hABCC2; CT | RR: 7.8 | Cui et al., 1999 |
| E3040-glucuronide | SD/EHBR Rat CMV | $K_m$: 5.7 μM | Niinuma et al., 1997 |
| Enalapril | OATP1B1/ABCC2-MDCKII OATB1B1-MDCKII; ME | ER: 3.59 ± 0.871; Ctrl OATP1B1: 0.374 ± 0.194 | Liu et al., 2006 |
| Epirubicin | HEK-hABCC2; CT | RR: 5 | Cui et al., 1999 |
| Ethinyl-estradiol-glucuronide | Sf9-ABCC2; VT | $K_m$: 35 ± 3.5 μM | Chu et al., 2004 |
| Etoposide VP-16 | Gli36-VP1; CT | RR: 128 | Matsumoto et al., 2005 |
| | HEK-hABCC2; CT | RR: 4 | Cui et al., 1999 |
| Grepfloxacine-glucuronide | SD/EHBR Rat CMV; VT | $K_m$: 7.2 μM | Sasabe et al., 1997 |
| GW1843 polyglutamate TS inhibitor | 2008/ABCC2 cells; CT | $IC_{50}$: 162.0 ± 32.0 μM; RR: 101.0 | Hooijberg et al., 1999 |
| Irinotecan CPT11 carboxylate form | SD/EHBR Rat CMV; VT | SD: ~1,500 pmol/mg protein/2 min; EHBR: None | Chu et al., 1997 |
| Irinotecan active metabolite: SN-38 carboxylate form | SD/EHBR CMV | Uptake: SD: ~1,500 pmol/mg protein/2 min; EHBR: ~100 pmol/mg protein/2 min | Chu et al., 1997 |
| Irinotecan active metabolite-Glu: SN38-glucuronide carboxylated form | SD/EHBR CMV; VT | Uptake: SD: ~650 pmol/mg protein/2 min; EHBR: ~100 pmol/mg protein/2 min | Chu et al., 1997 |
| Irinotecan active metabolite-Glu: SN38-glucuronide lactone form | SD/EHBR CMV; VT | Uptake: SD: ~500 pmol/mg protein/2 min; EHBR: ~50 pmol/mg protein/2 min | Chu et al., 1997 |
| Lopinavir LVR | MDCKII-ABCC2/MDCKII-WT; ME | ER: ABCC2: 2.9; WT: 1.32 | Li et al., 2008 |
| Methotrexate | CMV from SD/EHBR rat; VT | $K_m$: 296 ± 62 mM; not transported by EHBR | Masuda et al., 1997 |
| | 2008/ABCC2; VT and CT | Uptake: 0.6 ± 0.1 pmol/mg/min; $IC_{50}$: 15.2 ± 4.1 nM; RR: 1.1 | Hooijberg et al., 1999 |
| | Sf9/ABCC2; VT | $K_{1/2}$: 2.5–3 mM | Bakos et al., 2000 |
| Morphine-3-glucuronide | Sf9-ABCC2; VT | $K_m$: 50 ± 5 μM | van de Wetering et al., 2007 |
| Olmesartan | ABCC2-Sf9; VT | Uptake: 1,600 μL/mg prot /30 min | Nakagomi-Hagihara et al., 2006 |
| | LLC-PK1-ABCC2 | $K_m$: 14.9 ± 5.39 μM | Yamada et al., 2007 |
| Paclitaxel | MDCKII-ABCC2/Neo; ME | ER: ABCC2: 9.0 ± 0.6 Neo: 1.4 ± 0.1 | Huisman et al., 2005 |
| Para-amino-hippuric acid PAH | HEK-hABCC2; VT | $K_m$: 880 μM | Leier et al., 2000 |
| | MDCKII-hABCC2; VT | $K_m$: 2,100 ± 600 μM | Smeets et al., 2004 |
| | Sf9-hABCC2; VT | $K_m$: 5,000 ± 2,000 μM | Smeets et al., 2004 |
| Pravastatin | MDCKII-OATP2/ABCC2; ME | $K_m$: 24.3 ± 10.4 μM | Sasaki et al., 2002 |
| | MDCKII-Oatp4/rAbcc2; ME | $K_m$: 48.3 ± 7 μM | Sasaki et al., 2004 |
| Saquinavir | MDCKII-ABCC2; ME | ER = 7.4; ER 1.3 in the presence of MK571 | Jain et al., 2008 |
| Gly-Val-saquinavir | MDCKII-ABCC2; ME | ER: 2.1; ER in the presence of MK571 = 1.2 | Jain et al., 2008 |
| Sulfinpyrazone | MDCKII-ABCC2/MDCKII; ME | ER: ABCC2: 2.03; ctrl: 1.17 | Evers et al., 2000 |
| Thromboxane A2 receptor antagonist, Z-335 | SD/EHBR Rat CMV; VT | $K_m$: 189 μM | Kawabata et al., 2004 |
| Tomudex ZD 1694 polyglutamate TS inhibitor | 2008/ABCC2 cells; CT | $IC_{50}$: 3.04 ± 1.24 μM; RR: 3.9 when S incubated for 1 hour | Hooijberg et al., 1999 |
| Topotecan | MDCKII/ABCC2; ME | $K_m$: >500 mM | Li et al., 2008 |
| Valsartan | LLC-PK1-ABCC2; VT | $K_m$: 30.4 ± 17.7 μM | Yamashiro et al., 2006 |
| Vinblastine | MDCKII-ABCC2/MDCKII; ME | $K_m$: 137.3 ± 33.6 mM | Tang et al., 2002 |
| | LLC-hABCC2; CT | RR: 7 | Kawabe et al., 1999 |
| | MDCKII-hABCC2; CT | RR: 2.4 | Cui et al., 1999 |

Table 4. continued on next page

**Table 4.** Continued.

| Compound | System | Computed values /*in vitro* kinetic parameters | References |
|---|---|---|---|
| Toxicants and toxicants conjugates | | | |
| 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine PhIP | MDCKII-ABCC2/MDCKII; ME | BA: ABCC2/Ctrl: 26.05 ± 2.2 nM/12.74 ± 1.23 nM | Dietrich et al., 2001b |
| Dinitrophenyl-S-gluthatione | SD/EHBR CMV; VT | Uptake: SD: 140 μL/mg prot./2 min; EHBR: 5 μL/mg prot./2 min | Masuda et al., 1997 |
| | MDCKII-ABCC2; VT | $K_m$: 6.5 μM | Evers et al., 1998 |
| Mercury: methylmercury-*N*-acetyl-L-cysteine | WR/TR$^-$ CMV; VT | Uptake: WT: ~0.15 pmol/mg prot./5 min; TR$^-$: ~0.04 pmol/mg prot./5 min | Madejczyk et al., 2007 |
| N-ethylmaleimide gluthatione | Sf9-ABCC2; VT | $K_{1/2}$: 2.5 mM | Bakos et al., 2000 |
| NNAL-glucuronide | HEK293T-ABCC2; VT | Uptake: 22 pmol/mg protein/15 min | Leslie et al., 2001 |
| Ocratoxin A | HEK-hABCC2; VT | Uptake: 1.2 nmol/mg prot./min at 200 μM S | Leier et al., 2000 |
| Fluorescent substrates | | | |
| Calcein | Sf9-ABCC2; VT | Uptake: 190 pmol/mg protein/20 min | Masereeuw et al., 2003 |
| CDF | Sf9-rAbcc2; VT | Uptake: ~1,100 pmol/mg protein/10 min | Zamek-Gliszczynski et al., 2003 |
| Fluo-3 | Sf9-ABCC2; VT | Uptake: 2,100 pmol/mg/20 min | Masereeuw et al., 2003 |
| Fluo-3 | WR/GY-TR$^-$ CMV; VT | Transport: WR: 154.6 ± 4.4 pmol · mg prot$^{-1}$ · min$^{-1}$ $K_m$: 3.7 μM; GY/TR$^-$: 11.1 ± 2.4 pmol · mg prot$^{-1}$ · min$^{-1}$ | Nies et al., 1998 |
| Sulfobromophthalein | Sf9-Abcc2; VT | $K_m$: 2.96 ± 0.91 μM rat; 5.35 ± 1.52 μM mouse; 4.82 ± 0.47 μM dog; 4.67 ± 1.67 μM monkey | Ninomiya et al., 2006 |
| Sulfobromophthalein | HEK-hABCC2; VT | $K_m$: 12 μM | Cui et al., 2001 |

BA, basal to apical transport; CT, cytotoxicity assay or growth inhibition assay; EF, efflux ratio BA/AB;
GY/TR-/EHBR, Abcc2-deficient rats;
ME, monolayer efflux assay;
RR, relative resistance over parental line;
S, substrate concentration;
SD, Sprague-Dawley rat;
VT, vesicular transport;
WR, Wistar rat.

positive and two electrostatically negative areas contributing as secondary sites. In contrast, Pedersen et al. (2008) suggested a model with three distinct sites, one cytosolic site for the small hydrophilic, negatively charged substrates, and a second cytosolic site for nontransported stimulators with similar molecular characteristics. A third site in the TM region was proposed for the more lipophilic cationic inhibitors that might partition into the lipid bilayer and are unlikely to bind to the former two sites because of charge repulsion.

In general, the above *in silico* approaches provide a 70–90% accuracy in predicting interactors and no-interactors, a value similar to what has been published for ABCB1 and ABCG2 (reviewed in Chang et al., 2006).

However, ABCC2 seems to be a more specific transporter, as only 22% of compounds tested were interactors (Pedersen et al., 2008), roughly half of what has been observed for ABCG2 (Matsson et al., 2007) and ABCB1 (Matsson et al., 2009). The substrate specificities of the three transporters are overlapping as two of three of the ABCC2 inhibitors interact with ABCB1 or ABCG2 (reviewed in Pedersen et al., 2008). This level of redundancy always highlights the importance of the respective function. ABCC2 is more specific than ABCC1, the third member of the MDR-ABC transporters. In a comparative study of ABCC1 and ABCC2, it was shown that the interaction of flavonoids with both transporters was dependent on the dihedral angle between the B- and C-ring of flavonoids (van Zanden et al., 2005). In addition, interaction with ABCC2, but not, ABCC1 exhibited a strong reliance on the presence of a flavonol B-ring pyrogallol group (van Zanden et al., 2005). Similarly, the nature and the magnitude of the ABCC2 transport and/or inhibition profiles were shown to be dependent on the torsion angles of the bi-phenyl system in a set of substituted heterocyclic compounds (Lai et al., 2007).

The *in silico* methods may provide information of affinities of transporter-ligand interactions, inasmuch as a 3D-QSAR model, developed by Hirono et al. (2005), was used to estimate binding affinity ($K_m$). In the study by Pedersen et al. (2008), compounds falsely predicted

as noninteractors had inhibition values very close to the cut-off value of 50% inhibition.

Finally, the most distinct groups among modulators and substrates are conjugates (e.g., glutathione, glucuronide, and sulfate) and unconjugated compounds. Despite the large number of unconjugated substrates, ABCC2/Abcc2 has always been featured as the transporter of conjugates (Konig et al., 1999). Indeed, $K_m$ values of the conjugates are in the high-nanomolar/low-micromolar range (Table 4). At the same time, $K_m$ values for the vast majority of unconjugated compounds are in the upper micromolar/lower millimolar range. The fluorescent substrates are exceptions, but a bias should be noted, as only substrates of significant affinity are used as fluorescent probes. Interestingly, the clear separation of affinities is not apparent among the modulators. However, the acidic form of SN-38 glucuronide has an about 20-fold greater affinity for the transporter than the unconjugated form, and an approximately 50-fold difference exists between the glutathione-conjugated and -unconjugated form of sulfobromophthalein, supporting the notion that negatively charged conjugate tags increase the affinity of compounds for ABCC2/Abcc2 (Table 3).

## Kinetics of ABCC2-mediated transport

Substrate-transport kinetics of most ABC-efflux transporters follows Michaelis-Menten kinetics. This is the case when a substrate binds to a single substrate-transport site within the transporter. ABCC2 was shown to have multiple binding sites; thus, substrates may bind to other, so-called modulator sites, besides the substrate-binding site. When the transport kinetics of such a substrate are investigated, the resulting curve does not fit to Michaelis-Menten kinetics. In 2003, two groups (Bodo et al., 2003; Zelcer et al., 2003) described that E2-17βG transport by ABCC2 does not follow Michealis-Menten kinetics. The substrate-concentration dependence of transport, rather, fits to a sigmoid model, explained by cooperativity. Interestingly, in the presence of a fixed concentration of a so-called modulator molecule (e.g., sulfanitran or indomethacin), the transport becomes Michealis-Menten. A possible explanation is that E2-17βG binds to two distinct binding sites of ABCC2, while the modulator competes with it on one of the sites, the modulator-binding site, shifting the concentration curve. When studying the effect of the modulator on the transport of low E2-17βG concentrations, one observes the stimulation of substrate transport, rather than inhibition.

Most studies use VT experiments to study the kinetics of E2-17βG transport by ABCC2. The origin of the vesicles can be native tissue (e.g., liver canalicular vesicles), stably transfected mammalian cells, or baculovirus-infected Sf9 cells. The cooperativity is observed in the case of the human and mouse transporter with disregard of the nature of the expression system (Bodo et al., 2003; Zelcer et al., 2003; Borst et al., 2006b; Ninomiya et al., 2006; Zimmermann et al., 2008). Abcc2-mediated E2-17βG transport in other species (e.g., the rabbit and cynomolgous monkey) was shown to follow Michaelis-Menten kinetics (Shilling et al., 2006; Yasunaga et al., 2008). There are controversial reports regarding the transport of E2-17βG in rats and dogs (Ninomiya et al., 2005; Borst et al., 2006b; Shilling et al., 2006). The cooperativity of E2-17βG transport was described in more complex systems (Heredi-Szabo et al., 2009). Our group (Heredi-Szabo et al., 2009) investigated the effect of modulators on E2-17βG transport in sandwich-cultured human and rat hepatocytes. Even though E2-17βG is a substrate of more than one canalicular transporter (i.e., MDR1 and BCRP besides ABCC2), the transport stimulation was clear in the presence of modulators, such as probenecid, indomethacin, or benzbromarone. Moreover, the biliary efflux of E2-17βG was stimulated by the above-mentioned molecules in bile-cannulated rats (Figure 2).

E2-17βG is an important substrate of ABCC2, being responsible for the biliary elimination of this cholestatic compound (Morikawa et al., 2000). The role of Abcc2 in E2-17βG-induced cholestasis was proven by administering the compound to normal Wistar and GY/TR$^-$ (Abcc2-deficient) rats (Huang et al., 2000). GY/TR$^-$ rats did not develop cholestasis, even when administering 10 times the dose, resulting in cholestasis in control animals. The mechanism behind the reduced taurocholate transport is a transinhibition of the Bsep transporter at the canalicular side (Stieger et al., 2000; Pauli-Magnus and Meier, 2005), that is, the characteristics of E2-17βG and progesterone metabolites.

E2-17βG is not the only substrate of ABCC2 where the phenomenon of transport cooperativity can be observed. Studies from the lab of Alfred Schinkel have shown that the transepithelial transport of several drug substrates of ABCC2, including docetaxel, paclitaxel, etoposide, vinblastine, saquinavir, ritonavir, and indinavir (Huisman et al., 2002, 2005; Lagas et al., 2009), was stimulated in the presence of, for example, probenecid. As these compounds are effective anticancer and -viral drugs, ABCC2 may play a role in their loss of efficacy and the development of resistance, despite the fact that this transporter is not expressed at the site of action for these drugs. Besides drug molecules, ABCC2-mediated transport of fluorescent dyes, such as Fluo-3, can be stimulated by modulator drugs (Heredi-Szabo, unpublished observation).

There are substrates of ABCC2 that bind only to the substrate-binding site and display Michaelis-Menten kinetics. These substrates include LTC4 (Kawabe et al., 1999; Jedlitschky and Keppler, 2002), bilirubin glucuronide (Jedlitschky et al., 1997; Kamisako et al., 1999),

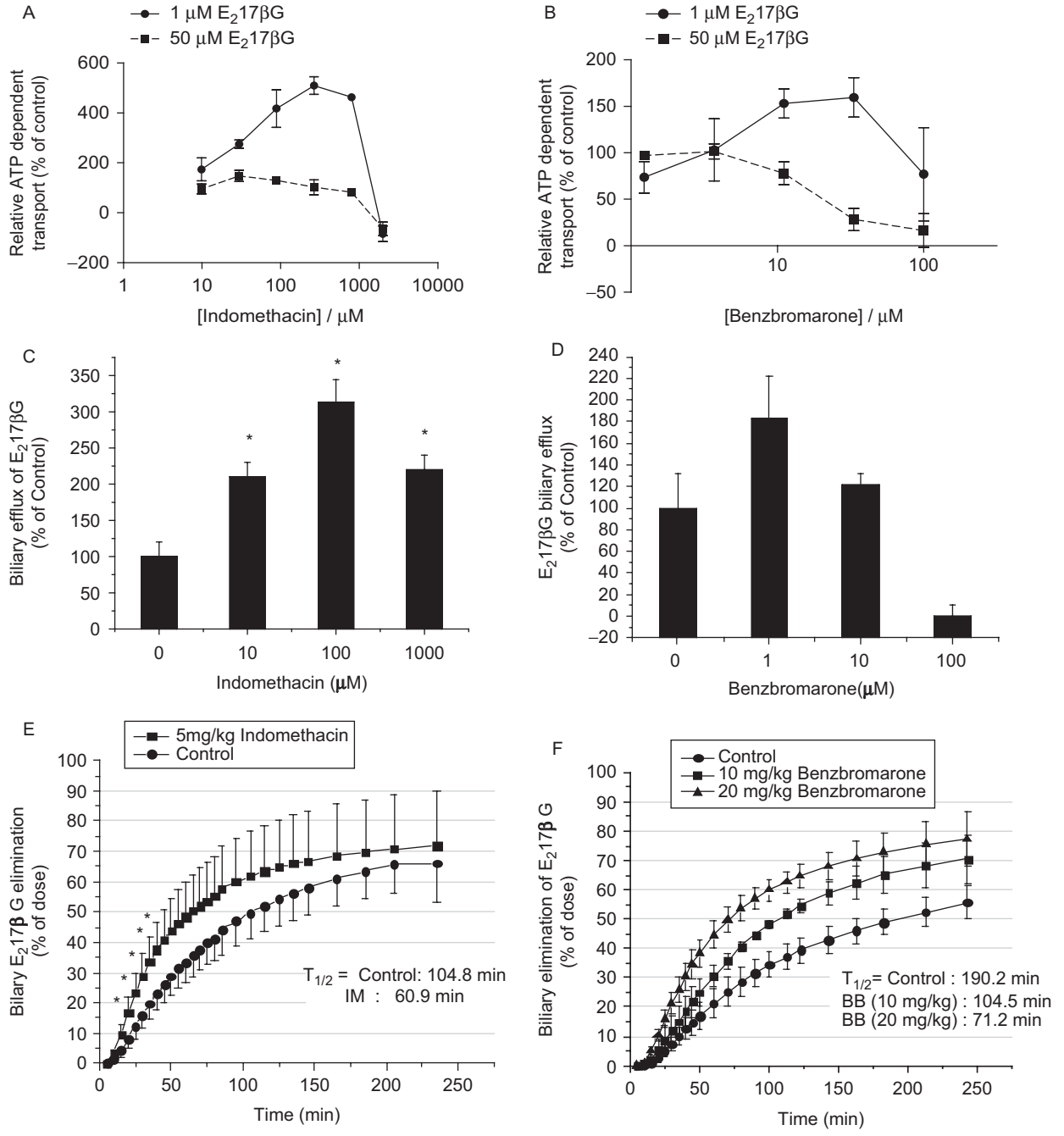


**Figure 2.** Effect of indomethacin (A, C, E) and benzbromarone (B, D, F) on rat Mrp2-mediated E2-17bG transport. Sf9 vesicles expressing rat Mrp2 (A, B), sandwich-cultured rat hepatocytes, and bile-cannulated rats were used for the determinations (Heredi-Szabo et al. 2009).

E2-3βG (Gerk et al., 2004), DNP-SG (Madon et al., 1997), and CDCF (Heredi-Szabo et al., 2008).

An example for the diverse nature of the ABCC2-binding pocket is methotrexate (MTX) transport. MTX does not, or only slightly, modulates E2-17βG transport (120–130%, compared to control) (Zelcer et al., 2003; Pedersen et al., 2008), probably binding to a distinct site of ABCC2. Renal elimination of MTX can be hindered by nonsteroidal anti-inflammatory drugs (NSAIDs), resulting in toxic levels of the folate antagonist in the plasma (Kremer and Hamilton, 1995). The two important transporters localized in the kidney proximal tubules transporting MTX are ABCC2 and ABCC4. MTX transport by these proteins follows Michaelis-Menten kinetics and can be inhibited by various NSAIDs (El-Sheikh et al., 2007; Nozaki et al., 2007). Even though there were huge differences in the inhibitory effects observed, both groups found that phenylbutazone stimulated ABCC2-

mediated MTX transport significantly, by around 70%. Also, the modulator potential of the same molecules (e.g., indomethacin, probenecid) differs, based on the identity of the substrate. A proof that these two substrates bind to different sites in the ABCC2-binding pocket is the characterization of $Trp^{1254}$ mutants (Ito et al., 2001a), as discussed earlier.

In summary, ABCC2/Abcc2 is a truly versatile transporter regarding its transport kinetics. Besides substrates that bind to only one binding site of the protein and whose transport follow Michaelis-Menten kinetics, one finds substrates binding to more than one binding site of ABCC2/Abcc2. The transport of these substrates, an example being E2-17βG, can be potentiated by drug molecules that, in the case of the liver, may result in cholestasis.

## Functions in various physiological and pharmacological barriers

ABCC2 was first cloned from hepatocytes (Buchler et al., 1996; Paulusma et al., 1996; Ito et al., 1997). Subsequently, it has been identified in different tissues of pharmacologically relevant species (reviewed in Bleasby et al., 2006). ABCC2 is expressed at major physiological barriers, such as kidney proximal tubular epithelial cells (Schaub et al., 1997; van Aubel et al., 1998; Schaub et al., 1999), enterocytes of the small and large intestine (Paulusma et al., 1996; Hinoshita et al., 2000; Mottino et al., 2000; van Aubel et al., 2000), and syncitiotrophoblast of the placenta (St-Pierre et al., 2000; Pascolo et al., 2003). Interestingly, ABCC2 protein expression has not been unequivocally shown in endothelial cells composing the blood-brain (Nies et al., 2004; Hoffmann et al., 2006), blood-retinal (Mannermaa et al., 2006), and blood-testis (Bart et al., 2004) barriers under physiological conditions. ABCC2 always localizes in the apical membranes, unlike some other members of the subfamily, such as ABCC4, that is in the apical membrane of kidney proximal tubule cells (van Aubel et al., 2002), and the basolateral membrane of hepatocytes (Rius et al., 2003), or ABCC1, that is thought to localize in the basolateral membrane of enterocytes (Peng et al., 1999; Prime-Chapman et al., 2004) and in the apical membrane of the brain microcapillary endothelial cells (Sugiyama et al., 2003; Nies et al., 2004), although data are conflicting on the latter (Roberts et al., 2008).

Multidrug resistance ABC (MDR-ABC) transporters play two major roles: an excretory and a barrier function. The most relevant systems to investigate these functions are *in vivo* studies using mutant and knock-out animals, isolated organs or cells, as well as vesicles derived from tissues from these animals. Data from studies addressing these functions were compiled (Table 5). Only studies where transporter identity is assured, either by using knock-out, mutant animals, tissues derived from these animals, or by performing complementary studies on vesicles, were cell lines with specific expression of ABCC2/Abcc2 included. The list of ABCC2/Abcc2 substrates contains different classes of molecules: endogenous compounds, drugs, toxic chemicals (i.e., organic and inorganic), and nutraceuticals as well as their conjugates.

Excretion is the only role ABCC2 plays in the hepatocyte. Studies on mutant rats as well as DJS patients have shown striking defects in biliary excretion of ABCC2 substrates, as discussed earlier.

In theory, it may play dual roles in the intestine, as it is active in pumping back conjugates formed in enterocytes, such as sulfate and glucuronide derivatives of flavonoids and other polyphenolic nutraceuticals (Jager et al., 2003; Williamson et al., 2007; Zhang et al., 2007), or drugs, such as ezetimibe (Oswald et al., 2006a, 2006b), into the intestinal lumen. The list is certainly much longer (reviewed in Brand et al., 2006; Ofer et al., 2006; Walle, 2007); however, in only a subset of studies were transporter-specific reagents (i.e., vesicles overexpressing ABCC2/Abcc2, or $Abcc2^{-/-}$ animals) used. ABCC2/Abcc2 was proposed to act as a barrier to reduce absorption of parent drugs or toxic compounds. To prove the hypothesis, several drugs and toxicants were administered orally to wild-type/control and mutant/knock-out animals and pharmacokinetic data determined. In one study, no significant difference was found in $F_{gut}$ values for MTX, probenecide and furosemide in EHBR, and control SD rats, whereas statistically significant differences were shown in biliary clearance for MTX and probenecide and 50% decrease for furosemide (Chen et al., 2003b). Interestingly, a 2.1-fold decrease in intestinal excretion of intravenous (i.v.)-administered MTX was found in $Abcc2^{-/-}$ mice, compared with controls (Vlaming et al., 2006). The role of Abcc2 in the absorption of orally administered pravastatin, another ABCC2/Abcc2 substrate drug, was addressed by using $TR^-$ and Wistar rats. The systemic exposure was increased to the same extent in the $TR^-$ animals after oral and intravenous (i.v.) administration. Therefore, it was concluded that intestinal Abcc2 does not play a significant role in pravastatin absorption (Kivisto et al., 2005). Similar findings were reported on grepfloxacine. Permeabilities measured in an Ussing chamber in secretory direction were 6-fold lower for intestinal tissue from EHBR rats vs. SD rats (Naruhashi et al., 2002). These correlated well with the 3–4-fold decrease in secretory clearance of i.v.-dosed drug in EHBR rats in the same study. No statistically significant difference was observed in the Ussing chamber experiment in the absorptive direction between the two strains (Naruhashi et al., 2002). Finally, the effect

**Table 5.** Pharmacokinetics of substrates affected by ABCC2/Abcc2.

| Compound | Assay | Data | References |
|---|---|---|---|
| Endogenous compounds | | | |
| E2-17-beta-Gluc | *In vitro* (CMV) and *in vivo* (biliary excretion after i.v.) experiments | Impaired in EHBR rats | Morikawa et al., 2000 |
| Hydroxy-nonenal-gluc | Biliary clearance | No detectable excretion in EHBR rats | Ji et al., 2002 |
| Drugs and drug metabolites | | | |
| Acetaminophen sulfate | IPL | Rate constant of biliary excretion was decreased by 90% in TR$^-$ rats, a further 50% decrease upon GF admin | Zamek-Gliszczynski et al., 2005 |
| Acetaminophen, sulfate, and glucuronide | IPL | A$^-$ sulf 20–30% in TR$^-$ A$^-$Gluc; A-GS negligible in TR$^-$ | Xiong et al., 2000 |
| Acetaminophen, glutathione, mercapturate, and glucuronide | Biliary excretion after i.v. administration | Biliary conc of A-GSH negligible; A-Gluc; A-NAC significantly reduced in TR$^-$ | Chen et al., 2003a |
| Acetaminophene glucuronide | Single-pass liver perfusion (wild type vs. TR$^-$) | 500-fold lower excretion rate in TR$^-$ livers | Xiong et al., 2002 |
| Azithromycin | Bolus injection, biliary excretion | 60% lower in EHBR rats (0.7 vs. 1.2% in SD) | Sugie et al., 2004 |
| Cefoperazone | Biliary and urinary excretion | Biliary excretion decreased by 90% in EHBR rats | Kato et al., 2008 |
| Cisplatin | *In vitro* and i.p. *in vivo* xenograft experiments | Lentiviral RNAi adm knocked down ABCC2 exp to cca 20%; cisp accumulation 3–4-fold incr; reduced $IC_{50}$ 4–5-fold; worked *in vivo* | Xie et al., 2008 |
| Diclofenac glucuronide | Liver perfusion (wild type vs. TR$^-$) | 30-fold lower excretion in TR- livers | Seitz et al., 1998 |
| [D-penicillamine2,5]enkephalin (DPDPE) | Sandwich hepatocytes | Biliary clearance decreased by 83% | Hoffmaster et al., 2005 |
| Doxorubicin | IPL | Biliary excretion decreased by 30% in TR$^-$ rats | Gaugg et al., 2001 |
| | *In vitro* (K562/ADR) *and in vivo* (biliary clearance) | Biliary excretion reduced in EHBR rats | Asakura et al., 2004 |
| | Clinical | Val1188Glu-Cys1515Tyr haplotype associated with acute anthracycline cardiotoxicity (ACT) | Wojnowski et al., 2005 |
| E3040 (6-hydroxy-5,7-dimethyl-2-methylamino-4-(3-pyridylmethyl) benzothiazole) and glucuronide | IPL and VT | In EHBR biliary clearance of E3040G was 1/30 of wild type | Takenaka et al., 1995 |
| Enalapril; enalaprilate | IPL | Biliary excretion reduced to almost zero in EHBR rat | Liu et al., 2006 |
| Ezetimibe and glucuronide | Intestinal secretion upon rifampicin treatm. | EG decreased in treated rats | Oswald et al., 2006a |
| | P.o. treatment intestinal secretion (EG content in feces) | EG decreased in TR$^-$ Lewis/1W rat | Oswald et al., 2006b |
| | Duodenal administration of ezetimibe; biliary excretion | 1.8-fold reduction in Abcc2$^{-/-}$ mice | de Waart et al., 2009 |
| Fexofenadine | Isolated perfused mouse liver | Biliary excretion rate decreased by 85% in the –/– mice vs. wild type | Tian et al., 2008 |
| | P.o. | 6-fold inc AUC and 2-fold brain/blood in P-gp k.o. mice; no effect on biliary Cl in EHBR rat | Tahara et al., 2005 |
| Gemfibrosil and glucuronides | Biliary clearance | G-Gluc excretion significantly decreased in TR$^-$ rats (22 vs. 83%) | Kim et al., 2003 |
| Grepafloxacin | Intestinal secretion using Ussing chamber (wild type vs. EISAI); i.v. adm and intestinal biliary secr | 6-fold decreased secretion in EISAI; 3-fold decrease in intestinal secretion and complete loss of grepfloxacine and metabolites in EHBR rats | Naruhashi et al., 2002 |
| Indinavir | Clinical; p.o. | MRP2-24C/T variant carriers had 24% faster indinavir oral clearance | Anderson et al., 2006 |
| Indomethacine (I); I-Gluc | i.v. and biliary excretion | I biliary Cl did not change, I-Gluc decreased by 50% in EHBR rats | Kouzuki et al., 2000 |

Table 5. continued on next page

**Table 5.** Continued.

| Compound | Assay | Data | References |
|---|---|---|---|
| Methotrexate | Infusion; collection of blood and bile samples (wild type vs. EISAI) | 39-fold reduced biliary clearance in EISAI | Chen et al., 2003b |
| | Elimination after i.v. administration in Mrp2$^{-/-}$ mice | 2.2-fold elevation in plasma levels; 2.1-fold decrease in intestinal content; 3.7-fold decrease in the intestinal wall; no difference in the liver | Vlaming et al., 2006 |
| | Biliary elimination | About a 20-fold decrease upon DPC333 administration in Wistar rats | Luo et al., 2007 |
| | P.o. | Increased $C_{max}$ (1.7-fold); AUC (1.7-fold); decreased Cl/F (1.6-fold) in EHBR | Naba et al., 2004 |
| | Biliary excretion after i.v. and plasma levels after oral administration | Decreased biliary clearance but no change in absorption in EHBR rats | Chen et al., 2003b |
| | Clinical; pediatric ALL | Plasma MTX 2-fold higher In female patients with at least one C-24T | Rau et al., 2006 |
| Methotrexate; MTX-OH | I.v. MTX | MTX AUC increased 2.0-fold, leading to 1.6-fold increase in urinary excretion in Abcc2$^{-/-}$; not seen in double k.o.; biliary excretion of MTX decreased 3.7-fold, but was unchanged in Abcc2; Abcc3–/; MTX-OH AUC increase 6.0 and 4.3-fold in Abcc2 and double k.o. and intestinal excretion significantly in Abcc2; Abcg2$^{-/-}$; biliary excretion of MTX-OH decreased 5.8-fold, but was unchanged in Abcc2; Abcc3$^{-/-}$ | Vlaming et al., 2008 |
| | I.v. MTX | MTX and MTX-OH AUC increased 2- and 6.2-fold in Abcc2$^{-/-}$ mice; biliary excretion decreased 23-fold and intestinal excretion significantly in Abcc2; Abcg2–/–; | Vlaming et al., 2009 |
| Mycophenolic acid | Clinical PK of p.o. administered MPA | ABCC2 C-24T polymorphism also seems to be associated with enhanced enterohepatic circulation of MPA | Miura et al., 2007 |
| Mycophenolic acid | Clinical, MRP2 C-24T and C-3972T | Protect renal transplant recipients from a decrease in MPA exposure associated with mild liver dysfunction. C-24T SNP is associated with a lower oral Cl of MPA | Naesens et al., 2006 |
| Mycophenolic acid glucuronide | Isolated perfused rat liver | Reduced by 90% in TR$^-$ rats | Westley, 2006 |
| Mycophenolic acid; glucuronide | Isolated perfused rat liver | No interaction with CsA; MPAG interacts with CsA in an MRP2-mediated manner | Hesselink et al., 2005 |
| | I.v. adm of MPA; biliary excretion of MPA and MPA-G | 70% decrease in biliary excretion of MPA and total elimination of MPAG in EHBR | Kobayashi et al., 2004 |
| | Clinical | Rifampin stimulated MPA glucuronidation and MRP2 expression | Naesens et al., 2006 |
| Oligodeoxynucleotides | Biliary elimination | Canalicular efflux lower in TR$^-$ rats | Lischka et al., 2003 |
| Olmesartan | I.v. administration; cannulation of bile duct | Bilary excretion was 6-fold lower in EHBR rats | Nakagomi-Hagihara et al., 2006 |
| Phenobarbital sulfate and glucuronide | IPL | In TR$^-$ cca 18% of P-sulf; P-Glu transport negligible | Patel et al., 2003 |
| Pravastatin | I.v. administration; PK | 6.1-fold increased AUC and 16.2-fold increased renal Cl in TR$^-$ rats | Kivisto et al., 2005 |
| Pravastatin | P.o. | Increased $C_{max}$ (4-fold); AUC (3.9-fold); decreased Cl/F (2.9-fold) in EHBR | Naba et al., 2004 |
| Pravastatin | P.o. clinical | ABCC2 c.1446C>G SNP reduced systemic exposure due to increased MRP2 expression | Niemi et al., 2006 |
| Probenecide | Infusion; collection of blood and bile samples (wild type vs. EISAI) | 37-fold reduced biliary clearance in EISAI | Chen et al., 2003b |

Table 5. continued on next page

**Table 5.** Continued.

| Compound | Assay | Data | References |
|---|---|---|---|
| Probenecide | I.v. administration, plasma conc+biliary efflux; oral admin plasma conc | Decreased biliary clearance, but no change in absorption in EHBR rats | Chen et al., 2003b |
| Rosuvastatin | I.v. administration; plasma conc and biliary efflux | A decrease of 53% in EHBR | Kitamura et al., 2008 |
| S-8921-Gluc | I.v. administration; biliary efflux | Reduced by 80% in EHBR | Sakamoto et al., 2008 |
| Telithromycin | I.v. administration; biliary efflux | Biliary clearance reduced 4.5-fold in EHBR rats | Yamaguchi et al., 2006 |
| Telmisartan-glucuronide | I.v. administration; cannulation of bile duct | Biliary excretion was 2-fold lower in EHBR rats | Nishino et al., 2000 |
| TR 14035 | Biliary excretion and total body excretion | Biliary and total body clearance decreased in EHBR rats | Tsuda-Tsukimoto et al., 2006 |
| Trabectedin | Isolated perfused rat liver | Biliary recovery decreased by 75% in $TR^-$ rats | Lee et al., 2008 |
| UCN-01 and glucuronides | IPL | Biliary excretion of UCN1 was reduced to 8.3% in $TR^-$ rats; glucuronide BE was reduced to 5.8–31% | Hagenauer et al., 2004 |
| Valproate-glucuronide | Single-pass liver perfusion (+/– probenecide treatment) | Probenecide inhibited excretion | Ward et al., 2001 |
| Valsartan | I.v. administration; cannulation of bile duct | $Cl_{bile}$ r educed 50-fold | Yamashiro et al., 2006 |
| Vincristine | *In vitro*; HepG2 | Adenoviral vectors of anti-ABCC2 antisense constructs; reduced $IC_{50}$ vincristine (50-fold) | Folmer et al., 2007 |
| (3S)-3-[(3R or 3S)-2-oxo-3--[3-(5,6,7,8-tetrahydro-1-,8-naphthyridin-2-yl)propyl] pyrrolidin-1-yl]-3-quinolin-3-ylpropanoic acid | I.v. administration; biliary excretion | Both isomers showed decreased biliary clearance in EHBR rats | Prueksaritanont et al., 2003 |
| Dipyrrinone sulfonates[a] | Biliary excretion | Decreased excretion into bile in $TR^-$ | McDonagh et al., 2002 |
| Nutrients, nutraceuticals, and their metabolites | | | |
| Baicalin | Efflux rate in everted jejunal sacks | 56% decreased in EHBR rats vs. SD | Akao et al., 2004 |
| Falvopiridol (FLAP) and glucuronides | IPL | FLAP-Gs dxecreased to 4.3 and 5.4% in $TR^-$ | Jager et al., 2003 |
| Flavonolignan-glucuronides (flavonolignans from silymarin) | Isolated perfused rat liver | Lower by 80–92% in $TR^-$ rats | Miranda et al. 2008 |
| 4-methylumbelliferone conjugates | Isolated perfused rat liver (Wistar, $TR^-$; +/– GF120918 | The glucuronide is almost completely excreted by Mrp2 | Zamek-Gliszczynski et al., 2006 |
| Resveratrol and conjugates | Isolated perfused rat liver | Resveratrol efflux dropped to 60%; efflux of conjugates decreased to 0–6% of wild type | Maier-Salamon et al., 2008 |
| Valerenic acid | Isolated perfused rat liver | Efflux of valerenic acid and conjugates in $TR^-$ rats dropped to 1–9% of wild types | Maier-Salamon et al., 2009 |
| Toxic compounds and their conjugants | | | |
| Bisphenol-glucuronide | ILP | Biliary efflux eliminated in EHBR rats | Inoue et al., 2005 |
| $CH_3HHg(+)$ complexed with DMPS or DMSA | I.v. administration; tissue, urine, and feces analyzed | Lower amount of methyl-mercury in urine and feces of $TR^-$ rats than in wild type | Zalups and Bridges, 2009 |
| $CH_3HHg(+)$ complexed with N-acetyl-cys | I.p. administration and urine and feces analysis | About 70% decrease in renal excretion in $TR^-$ rats | Madejczyk et al., 2007 |
| Demethyl-phalloidin | *In vivo* biliary excretion | A 59% decrease in $TR^-$ rats | Gavrilova et al., 2007 |
| IQ (2-amino-3-methylimidazo[4,5-f]quinoline) | Plasma levels after p.o. administration in $Mrp2^{-/-}$ mice | 1.7-fold increase | Vlaming et al., 2006 |
| Micafungin | I.v. administration; biliary efflux | Reduced by 60% in EHBR | Abe et al., 2008 |
| PhIP | Plasma levels after p.o. administration in $Mrp2^{-/-}$ mice | 1.9-fold increase | Vlaming et al., 2006 |
| | PK | 2-fold absorption in $TR^-$ rats (plasma levels 2-fold higher) | Dietrich et al., 2001b |

Table 5. continued on next page

**Table 5.** Continued.

| Compound | Assay | Data | References |
|---|---|---|---|
| Fluorescent compounds and their conjugants | | | |
| Calcein | Isolated kidney perfusion | Calcein excretion was significantly reduced in TR$^-$ | Masereeuw et al., 2003 |
| CDCF | Single-pass liver perfusion | Biliary clearance negligible vs. 65% in wild type | Nezasa et al., 2006 |
| | Hepatocyte sandwich culture | No excr of CDCF into canaliculi | Zamek-Gliszczynski et al., 2003 |
| DBSP | PK parameters of i.v. DBSP in Mrp2$^{-/-}$ mice | 1.9-fold increased AUC; 2.8-fold decreased Cl | Chu et al., 2006 |
| Dipyrrinone Sulfonate | Liver perfusion (wild type vs. TR$^-$) | Lower excretion in TR$^-$ livers | McDonagh et al., 2002 |
| DNP-SG | I.v. administration of CDNP; biliary efflux of DNP conjugates | Elimination of biliary DNP-SG excretion in EHBR rats | Gotoh et al., 2000 |
| Fluo-3 | Isolated kidney perfusion | Fluo-3 excretion was significantly reduced in TR$^-$ | Masereeuw et al., 2003 |
| Phenolphthalein-sulfate | Biliary excretion | Biliary elimination delayed and decreased in EHBR rats | Tanaka et al., 2003 |
| Phenosulfophtalein (PSP) | I.v. administration; biliary efflux | $CL_{bile}$ decreased by 60% in EHBR rats | Itagaki et al., 2003 |

i.v., intravenous; i.p., intraperitoneal; IPL, liver perfusion; CL, clearance; DBSP, dibromosulfophthalein.
[a]8-(2-ethanesulfonic acid)-3-ethyl-2,7,9-trimethyl-1,10-dihydro-11H-dipyrrin-1-one (xanthosulfonic acid) and a fluorescent analogue (8-desethyl-N,N'-carbonyl-kryptopyrromethenone-8-sulfonic acid).

of Abcc2 on ADME properties of 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP) was studied with a similar strategy, leading to a similar conclusion: a 1.8-fold decrease in permeability of PhIP through EHBR intestinal samples in the secretory direction and no significant change in the absorptive direction (Dietrich et al., 2001b). The same group showed that Abcc2, indeed, facilitates PhIP secretory transport, as direct intestinal excretion of the unmetabolized compound was 3 times higher in Wistar than in TR$^-$ rats (Dietrich et al., 2001a). Altogether, ABCC2/Abcc2 seems to affect the intestinal permeability of its substrates in the secretory, but not in the absorptive, direction.

In the kidney, ABCC2 could actively promote secretion and inhibit the reabsorption of substrates. Little information is available on the effect of ABCC2/Abcc2 on the renal excretion of drugs. Perhaps, this can be explained by the elevated expression of ABCC4 in TR$^-$ (Chen et al., 2005) and EHBR rats as well as in Abcc2$^{-/-}$ mice (Chu et al., 2006; Vlaming et al., 2006). Abcc4 has a substrate specificity that greatly overlaps the substrate specificity of Abcc2 (Keppler, 2005; El-Sheikh et al., 2007) and likely compensates for Abcc2 function on common substrates. The fact that decreased urinary excretion in TR$^-$ rats was observed in perfusion experiments on isolated kidneys, using calcein as well as Fluo3, two Abcc2 substrates that are not transported by Abcc4 that support this notion (Masereeuw et al., 2003).

Abcc2-dependent renal excretion of N-acetyl-cysteine (Madejczyk et al., 2007) as well as 2,3-dimercaptopropane-1-sulfonic acid and meso-2-,3-dimercaptosuccinic acid complexes (Bridges et al., 2008; Zalups and Bridges, 2009) of inorganic mercury (Bridges et al., 2008) and methylmercury (Madejczyk et al., 2007; Zalups and Bridges, 2009) have been reported. Renal specificity was provided by Oat1 and Oat3 (Koh et al., 2002).

In placenta, ABCC2/Abcc2 is expressed in the apical membrane of syncytiotrophoblast cells (St-Pierre et al., 2000; Aye et al., 2007). It has been suggested that it transports, and thus regulates, placental levels of endothelin-1 (St-Pierre et al., 2000) and takes part in effluxing potentially toxic cholephilic organic anions produced by the fetus (Marin et al., 2005). To date, no studies employing Abcc2$^{-/-}$ animals have been published to substantiate these suggestions.

Mice null for multiple transporters allow for elegant studies on transporters' cooperation and complementation. Pharmacokinetic studies showed that in Abcc2$^{-/-}$ mice, the plasma levels of MTX and its metabolite, 7-hydroxy-MTX (Vlaming et al., 2008), as well as morphine-3-glucuronide (van de Wetering et al., 2007), were higher than in wild-type animals. On the other hand, in Abcc2, Abc3 double-knock-out mice, the plasma levels were the same (Vlaming et al., 2008) or lower (van de Wetering et al., 2007). This proves that Abcc2 plays an important role in biliary elimination of these substrates and also shows that sinusoidal transport by Abcc3 in Abcc2$^{-/-}$ animals is efficient. Therefore, Abcc2 and Abcc3 are alternative routes for the transport of MTX and its toxic metabolite, 7-hydroxy-MTX (Vlaming et al., 2008), as well as morphine-3-glucuronide (van de Wetering et al., 2007).

Abcc2 and Abcg2 have overlapping substrate specificity, as both transporters have organic anions, glucuronate, and sulfate conjugates among their substrates. In addition, these transporters reside in the same (i.e., apical) membrane in the hepatocytes, enterocytes, and

kidney proximal tubule cells (reviewed in Leslie et al., 2005), making them perfect candidates for transporter complementation. In Abcc2 knock-out mice, biliary exretion of MTX, 7-hydroxy-MTX (Vlaming et al., 2008) and ezetimibe glucuronide (de Waart et al., 2009) significantly decreased. The decrease in the biliary excretion in the Abcg2$^{-/-}$ was small, statistically not significant for MTX and 7-hydroxy-MTX (Vlaming et al., 2009), and no change, compared to the wild type, was observed for ezetimibe glucuronide (de Waart et al., 2009). However, a further decrease was observed for all three substrates when Abcg2 was knocked out on the Abcc2$^{-/-}$ background. Interestingly, an increased urinary elimination of MTX and 7-hydroxy-MTX was observed in Abcc2$^{-/-}$ mice (Vlaming et al., 2009), whereas a significantly decreased urinary elimination of both substrates was seen in Abcg2$^{-/-}$ mice, when compared to the Abcc2$^{-/-}$ mice, despite similarly elevated plasma levels of MTX and 7-hydroxy-MTX in both strains (Vlaming et al., 2009), suggesting that in a wild-type situation, Abcg2 is the dominant transporter of MTX and 7-hydroxy-MTX in the kidney.

Abcb1 also colocalizes with Abcc2 in hepatocytes, enterocytes, and kidney proximal tubule cells (reviewed in Leslie et al., 2005). Yet, the overlap of the substrate specificities of the two transporters is limited, as Abcb1 mostly transports hydrophobic compounds and cations and Abcc2 prefers anionic compounds. Nonetheless, there are common substrates, such as paclitaxel (Huisman et al., 2005). The area under the concentration-time curve (AUC) of i.v.-administered paclitaxel in both Abcc2$^{-/-}$ and Abcb1a/1b$^{-/-}$ mice was 1.3-fold higher than in wild-type mice, and in the triple knock-out mice, it was 1.7-fold greater (Lagas et al., 2006). As far as the overall pharmacokinetics of paclitaxel is concerned, Abcb1a, Abc1b, and Abcc2 displayed complementary functions. Abcc2 dominated the hepatobiliary excretion, while Abcb1a and Abc1b dominated the direct intestinal excretion, with a minor role for Abcc2 in the intestine (Lagas et al., 2006). The $AUC_{oral}$ of paclitaxel was 8.5-fold increased in Abcb1a/1b knock-outs, but was not affected by Abcc2 deletion. On an Abcb1a/1b$^{-/-}$ background, however, Abcc2 deficiency modestly increased the $AUC_{oral}$ (Lagas et al., 2006).

Fexofenadine, another common substrate of Abcb1a, Abc1b (Cvetkovic et al., 1999), and Abcc2 (Tian et al., 2008), displayed a similar pattern in mice, with Abcb1a/b playing the major role in limiting intestinal absorption (Tahara et al., 2005) and Abcc2 dominating the biliary excretion (Tian et al., 2008) of the drug.

Altogether, in the canalicular membrane, Abcc2 dominates other transporters in the transport of common substrates, such as conjugates, acidic drugs, as well as some hydrophobic compounds. In contrast, it is playing a minor role in the intestine and the kidney.

## ABCC2 and multidrug resistance

ABCC2 is present in a wide range of cancers, such as hepatocellular (Nies et al., 2001; Zollner et al., 2005), renal clear-cell (Schaub et al., 1999; Sandusky et al., 2002), colorectal (Hinoshita et al., 2000; Sandusky et al., 2002), ovarian (Arts et al., 1999; Sandusky et al., 2002), leukemias (van der Kolk et al., 1998; Laupeze et al., 2002; Steinbach et al., 2003), mesothelioma (Soini et al., 2001), lung (Young et al., 1999, 2001), breast (Sandusky et al., 2002; Faneyte et al., 2004), bladder (Tada et al., 2002), and gastric (Sandusky et al., 2002) cancer.

Significance of expression was shown in a study on hepatoid adenocarcinomas. The positive rates of ABCC2 (100 vs. 43.8%) were significantly higher in hepatoid than in control adenocarcinoma (Kamata et al., 2008). It was suggested that ABCC2, along with ABCC1 and ABCC6, might be responsible for the higher level of MDR in hepatoid adenocarcinomas than the control adenocarcinomas.

*In vitro* evidence was provided from using transfected and selected cells. Human ABCC2 overexpressed in HEK-293 cells resulted in resistance to substrate drugs etoposide, cisplatin, doxorubicin, and epirubicin (Cui et al., 2001). Further, cisplatin-selection pressure led to upregulation of ABCC2 in various human tumors (Taniguchi et al., 1996), including hepatocellular carcinoma cells (Wakamatsu et al., 2007) and non-small-cell lung cancer cells (reviewed in Nishio et al., 1999). In cisplatin-resistant cell lines, decreased accumulation of cisplatin was demonstrated (Taniguchi et al., 1996; Nishio et al., 1999). ABCC2 acts in concert with GSTP1 to confer cisplatin resistance. A correlation between GSTP1 overexpression and decreased cisplatin sensitivity has been published (Oguri et al., 2000). Cisplatin is detoxified by GSTP1 through glutathione adduct formation (Goto et al., 1999; Rudin et al., 2003). Glutathione-conjugated platinum compounds are quickly removed from cells, likely by ABCC2 (Harpole et al., 2001). The cooperation between ABCC2 and GSTP1 may be reserved for platinum-based chemotherapeutic drugs, such as in RCO.1, a 9-nitro-campthotecin selected prostate cancer cell line where ABCC2 upregulation was accompanied with downregulation of GSTP1 (Annereau et al., 2004). In general, resistance to platinum compounds is usually associated with increased ABCC2 expression, and knocking down ABCC2 by various methods increased drug accumulation and restored susceptibility to the drug. Reduction of ABCC2 by antisense DNA in rat (Itoh et al., 2002) and human (Folmer et al., 2007) hepatoma cells, as well as by shRNA vectors in the A2780/cp ovarian cancer cell line (Ma et al., 2009), increased the intracellular concentration of cisplatin. Reversal of cisplatin resistance by anti-ABCC2 antisense cDNA in the human hepatoma cell line (Koike et al., 1997) by shRNA in

ovarian (Ma et al., 2009) and nasopharyngeal carcinoma cell lines (Xie et al., 2008) or hammerhead ribozymes (Materna et al., 2005) in various cancer cells has been demonstrated *in vitro*.

*In vivo* relevance of ABCC2-mediated drug resistance was shown with xenotransplanted nasopharyngeal carcinoma (Xie et al., 2008) and hepatoma cells (Folmer et al., 2007), employing an antisense strategy.

A 25-fold decrease in $IC_{50}$ for cisplatin in ABCC2 antisense-transfected HepG2 cells correlated with a similar decrease of $IC_{50}$ values for doxorubicin (12-fold), vincristine (50-fold), and etoposide (25-fold), and relevance was shown for vincristine *in vivo* (Folmer et al., 2007). Nevertheless, data on platinum compounds dominate the field. Perhaps, the true nature of ABCC2 as a conjugate export pump shows once again.

Clinical data on significance of ABCC2 in MDR are even scarcer. Data of this kind usually come from pharmacogenomics studies. The issue is complicated by the fact that partially or completely nonfunctional variants would affect the overall exposition to the drug (i.e., increased AUC), making the function of ABCC2 expressed in the cancerous tissue hard to evaluate. The ABCC2 variant investigated most thoroughly is the C-24T change in the ABCC2 promoter. This variant confers an approximately 60% decrease in promoter activity (Choi et al., 2007) and higher plasma concentration of ABCC2 substrate drugs, such as telmisartan (Miura et al., 2009) and MTX (Rau et al., 2006), as well as lower oral clearance of mycophenolic acid (Naesens et al., 2006), was observed. In addition, the ABCC2-24T genotype was associated with higher response rate and longer progression-free survival in irinotecan-treated non-small-cell lung cancer patients (Han et al., 2007). More important, the C-24T change in ABCC2 increased platinum-based chemotherapy response (Sun et al., 2009).

Despite these data, ABCC2 is not considered an MDR-ABC transporter (Eckford and Sharom, 2009). Localization of ABCC2 in cancer cells adds another layer of complexity. ABCC2 is localized in the apical membranes of polarized cells. Upon the loss of cell polarization, ABCC2 quickly redistributes into intracellular membranes. It is conceivable that, at least in some cancer cells, ABCC2 mislocalizes. ABCC2 is expressed in a polarized fashion in the apical domain in human hepatocellular carcinoma (Nies et al., 2001). In a gallbladder carcinoma cell line, Mz-Cha1, 10% of cells exhibit ABCC2 expression in apical vesicular-like structures, but 90% of the Mz-Cha-1 cells grow in a rather unpolarized fashion, lacking these ABCC2-containing vacuoles (Rau et al., 2006). Similarly, clear-cell carcinomas, originating from the proximal tubule epithelium, express ABCC2 in 95% of cases. However, immunofluorescence microscopy showed a lack of a distinct apical-to-basolateral tumor-cell polarity and an additional localization of ABCC2 on intracellular membranes (Schaub et al., 1999). In addition, frequently aberrant localization was noted in primary ovarian cancer and expression levels were not correlated with clinical response to platinum-based chemotherapy (Guminski et al., 2006). Another study reported nuclear membraneaous localization of the protein in ovarian carcinoma samples (Surowiak et al., 2006). Nuclear localization was associated to adverse response to first-line chemotherapy as well as poor prognosis. In ovarian carcinoma cell lines, defective localization correlated with cisplatin resistance. Tissue microarrays revealed that nuclear-membrane localization inversely correlated with differentiation (Surowiak et al., 2006).

## Conclusions

Major ABC transporters colocalized in the apical membranes play dual functions: an excretory and a barrier function. Interestingly, in the case of ABCC2, the excretory role is dominant. Excretion is the only role ABCC2 plays in the hepatocyte, the barrier where ABCC2 function is most evident.

The broad substrate specificity of ABCC2 underscores its multifunctional nature. However, a closer look at the data generated on knock-out and mutant animals shows that the real physiological function is biliary transport of phase II conjugates. At the level of kinetics, it appears as higher affinity for these conjugates.

Individual methods, either *in vitro* or *in vivo*, may shed light on distinct aspects of transporter function. But, only approaches integrating methods of different complexities can address mechanistic, as well as relevance, aspects of transporter function.

## Declaration of interest

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