Review

Role of multidrug resistance associated proteins in drug development

Shu-Feng Zhou*

School of Health Sciences, RMIT University, Bundoora, Victoria, Australia.

ABSTRACT: The multidrug resistance associated proteins (MRP1, MRP2, MRP3, MRP4, MRP5, MRP6, MRP7, MRP8 and MRP9) belongs to the ATP-binding cassette superfamily (ABCC family) of transporters expressed differentially in the liver, kidney, intestine and blood-brain barrier. MRPs transport a structurally diverse array of endo- and xenobiotics and their metabolites (in particular conjugates) and are subject to induction and inhibition by a variety of compounds. An increased efflux of natural product anticancer drugs and other anticancer agents by MRPs in cancer cells is associated with tumor resistance. These transporting proteins play a role in the absorption, distribution and elimination of various compounds in the body. There are increased reports on the clinical impact of genetic mutations of genes encoding MRP1-9. Therefore, MRPs have an important role in drug development, and a better understanding of their function and regulating mechanism can help minimize and avoid drug toxicity, unfavourable drug-drug interactions, and to overcome drug resistance.

Keywords: MRP, Drug development, Single nucleotide polymorphism, Toxicity, Pharmacokinetics, Blood-brain barrier, Biliary excretion, Intestinal absorption, Drug transport

1. Introduction

The human body is continuously exposed to a great variety of xenobiotics *via* food, drugs, occupation and environment. Evolution has equipped the body with a plethora of protecting systems to defend itself against the potentially harmful effects of these compounds. One of the important and clinically relevant defense mechanisms include the active extrusion of xenobiotics by commonly shared transport proteins, mainly located in kidney, liver and intestine. The ATP-binding cassette (ABC) superfamily of transporters consist of a large number of functionally diverse transmembrane proteins which have been subdivided into seven families designated A through G (*1-4*). Members of this transport superfamily display high amino acid similarity of the 200 amino acids surrounding the ATP-binding folds. Approximately 1,100 ABC transporters are known at this time. Traffic ATPases and P-glycoproteins (PgPs) are other names used for this family. The family includes bacterial transporters, the cystic fibrosis transmembrane conductance regulator, the *Plasmodium falciparum* drug-resistance gene, and genes apparently involved in peptide transport during antigen presentation.

In humans, members of this family serve a variety of physiological roles in transmembrane transport and cell signalling, many of which are associated with disease phenotypes such as multidrug resistance, cystic fibrosis, Tangier disease, adrenoleukodystrophy and Zellwegers' syndrome (*1,2,4*). The available outline of the human genome contains 48 ABC genes (*5*); 16 of these have a known function and 14 are associated with a defined human disease (*6*). ABC transporters that pump cytotoxic drugs from the cell are also present in micro-organisms, and this is one of the main mechanisms by which pathogenic species can resist antibiotic treatment. The human family of ABC transporters includes at least 48 members with 7 subfamilies (*4*). They facilitate unidirectional translocation of chemically diverse substrates including amino acids, lipids, inorganic ions, peptides, saccharides, metals, drugs, and proteins. Energy derived from the hydrolysis of ATP is used to transport the substrate across the membrane against a concentration gradient (*7*). These transporters are present in almost all tissues and cell types in different amounts. A typical ABC transporter is characterized by the presence of three peptide motifs: *Walker A* and *B* sequences and the so-called ABC-signature sequence ("ALSGGQ") (*1,8*). Most ABC proteins from eukaryotes encode full transporters, consisting of two ATP-binding domains and 12 membrane-spanning regions or half transporters,

**Correspondence to:* Dr. Shu-Feng Zhou, School of Health Sciences, RMIT University, Bundoora, Victoria 3083, Australia;
e-mail: Shufeng.zhou@rmit.edu.au

which are presumed to dimerize (*9*). The MRP family contains at least nine members (MRP1-9, ABCC1-6 and ABCC10-12, respectively) with sizes from 1,325 to 1,545 amino acids. This probably completes the family, as there are no other putative MRP genes among the 52 human ABC transporter genes. ABCC7 (CFTR) is a chloride channel, and channels are not transporters. ABCC8 and 9 (SUR1 and 2), the sulfonylurea receptors, are the ATP-sensing subunits of a complex potassium channel and are not known to transport any substrates. The MRPs, CFTR, and the SURs are considered to evolve from a common ancestor, and these proteins are now grouped together in the C branch of the ABC transporter family. This paper highlights the pharmacological roles of MRPs and their implications in drug development.

2. Topology of MRPs

PgP/MDR1 consists of 1,276 to 1,280 amino acids with a molecular mass of 170 kDa. The commonly accepted model for the topologic structure of PgP has a tandemly duplicated structure, with each half of the molecule contains a nucleotide-binding domain (NBD) and reveals six predicted and highly hydrophobic transmembrane regions (*4*). The *N*- and *C*-termini, as well as the NBDs, are located intracellularly, and the first extracellular loop is *N*-glycosylated. Both NBDs are essential for proper functioning of the protein. Each consists of two core consensus motifs referred to as the *Walker A* and *B* motifs and a *S* signature of ABC transporters (*10*). These motifs generally are found in a wide range of ATPases, and they are involved directly in the binding and hydrolysis of nucleotides. Structures of bacterial ABC transporter proteins suggest that the two NBDs form a common binding site where the energy of ATP is harvested to promote efflux through a pore that is delineated by the transmembrane helice (*11*). The two half molecules are separated by a highly charged "linker region" which is phosphorylated at several sites by protein kinase C. Different topologic orientations of PgP have been reported, and several studies have indicated that conformational changes in the structure of PgP are involved in the mechanism of substrate efflux (*12*).

Like PgP, MRPs belong to ABC transporter superfamily. All MRP members have 2 hydrophobic transmembrane domains (TMD1 and TMD2) and 2 cytoplasmic NBDs (Figure 1) (*13*). The NBDs are responsible for the ATP binding/hydrolysis that drives drug transport, and their structure is conserved independently of the degree of primary-sequence homology (*14*). The TMDs contain the drug-binding sites that are likely located in a flexible internal chamber that is sufficiently large to accommodate different drugs. MRPs can be categorized according to the presence or absence of a third (NH_2-terminal) membrane-spanning domain (TMD_0) in their structure (Figure 1) (*15-17*). This topological feature can be found in MRP1, MRP2, MRP3, MRP6, and MRP7, while it is not possessed by MRP4, MRP5, MRP8, and MRP9 (*18-21*). TMD_0 is not essential for catalytic function or intracellular routing; the function of this domain is unknown (*22*). MRPs with this structural feature have the ability to transport conjugates, while MRPs without it are able to transport cyclic nucleotides. Long MRPs share an L_0 segment (Figure 1) with a highly conserved sequence near its *N* terminus. This sequence is also present near the *N* terminus of the short MRPs. It is essential for function and appears to associate with the membrane.

3. Substrate specificity, resistance profiles and inhibitor selectivity of MRPs

The first member of MRP family, MRP1 (ABCC1), was found in 1992 in lung cancer cell line conferring resistance to doxorubicin which was not related to PgP (*23*). The genes encoding MRP1 and PgP are evolutionarily very distant, and the primary structure of the two proteins is quite dissimilar, sharing only 15 percent amino acid identity (*23*). Most of the sequence similarity between MRP1 and PgP is found within the nucleotide-binding domains that generally are conserved among members of the ABC superfamily (*24*). MRP1 is larger than other full-length ABC proteins, containing approximately 250 additional amino acids in its NH_2-terminal. Thus, in addition to the 12 transmembrane segments characterizing PgP, MRP1 has five transmembrane domains. MRP1 is nearly present in all major tissues and in all peripheral blood cell types (*25,26*). The expression levels of MRP1 are different in various organs and cell lines (*27-30*). Natural product drugs such as vincristine, etoposide and doxorubicin are substrates for MRP1 (*31*). Although MRP1 and PgP have some identical substrates, they show difference in the substrate specificity. PgP can transport drugs in original form, while MRP1 can transport glutathione (GSH), oxidized GSH (GSSG), as well as a number of GSH, glucuronate and sulfate conjugates of drugs (Figure 2) (*31-33*). Additionally, MRP1 has several physiologic substrates, such as 17-β-D-estradiol-glucuronide ($E_2$17βG), the GSH-conjugated cysteinyl leukotriene C_4 (LTC_4), sulfated bile acids, prostaglandin (PG) A GSH conjugates, and unconjugated bilirubin (*34-39*). The high affinity for LTC_4 is a specific feature of MRP1, which may contribute to the distinguished role of MRP1 in immune responses associated with cellular excretion of LTC_4 (*40,41*). In contrast, PgP shows poor resistance to these conjugated organic anions (*32*). Moreover, significant species difference in the substrate specificity of MRP1 has been noted.

Substrates of MRP1 also include neutral and basic cytotoxic compounds without conjugation with GSH or other anionic drugs (*42,43*). However, intracellular GSH is needed when MRP1 transports these chemicals (*44,45*). GSH concentrations increase in some organs of *mrp*

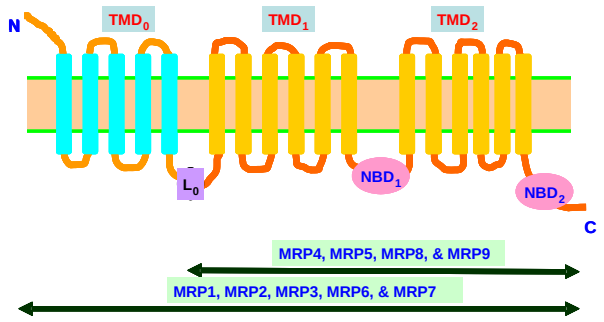

Figure 1. Predicted topological structure of MRP1-9 (ABCC1-6 and 10-12). All MRP members have two hydrophobic transmembrane domains (TMD1 and TMD2) and two cytoplasmic nucleotide binding domains (NBDs) responsible for the ATP binding and hydrolysis that drives drug transport. MRPs can be categorized according to the presence or absence of a third (NH_2-terminal) membrane-spanning domain (TMD_0) in their structure. This topological feature can be found in MRP1, MRP2, MRP3, MRP6, and MRP7, while it is not possessed by MRP4, MRP5, MRP8, and MRP9. TMD_0 is not essential for catalytic function or intracellular routing; the function of this domain is unknown. Long MRPs share an L_0 segment with a highly conserved sequence near its N terminus. This sequence is also present near the N terminus of the short MRPs. It is essential for function and appears to associate with the membrane.

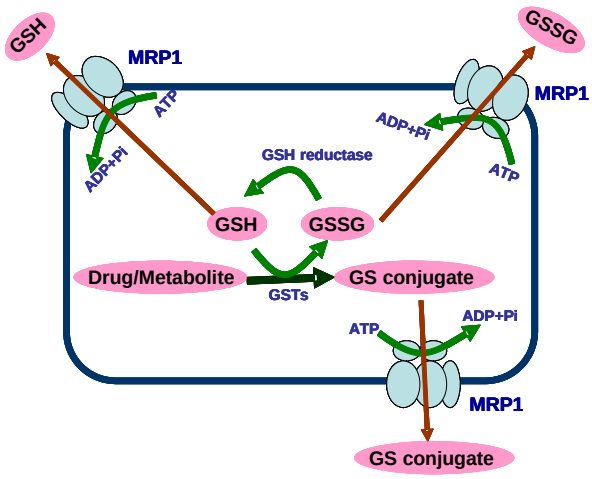

Figure 2. Glutathione (GSH)-dependent transport of drugs and their GSH conjugates by MRP1. P-glycoprotein can transport drugs in original form, while MRP1 can transport GSH, oxidized GSH (GSSG), as well as a number of GSH, glucuronate and sulfate conjugates of drugs. However, MRP1 has low affinity to GSH and GSSH. GSH not only enhances MRP1-mediated transport of hydrophobic xenobiotics, but also certain hydrophilic conjugated endobiotics, which represents a major detoxifying pathway.

knockout mice (*46*), and decrease in cells overexpressing MRP1 (*32,47*). MRP1 may reduce the harm of xenobiotics to cells by co-transporting the xenobiotics and GSH out (*46*). Overexpression of MRP1 is associated with an increased transport activity of compounds conjugated with GSH, glucuronide, or sulfate, which is known as glutathione conjugate pumps (*36,48,49*). GSH not only can enhance MRP1-mediated transport of hydrophobic xenobiotics, but also certain hydrophilic conjugated endobiotics (*30*). However, MRP1 has low affinity to GSH (*50,51*). Drugs including verapamil and apigenin have been demonstrated to increase the affinity of MRP1 to GSH (*52,53*). Vincristine uptake is inhibited by vinblastine but not daunorubicin or doxorubicin. Although GSH or vincristine alone has little effect on the MRP1-mediated transport of LTC_4, the combination of them becomes the potent inhibitor of MRP1-mediated transport of LTC_4 (*50*).

Human MRP1 confers resistance to anthracycline drugs, while Mrp1 from other species do not (*54,55*). Unlike PgP, however, MRP1 appears to cause resistance to some heavy metal ions, including arsenite and antimonials (*56,57*), which is consistent with the extensive homology of *MRP1* with the *Leishmania* arsenite transporter-encoding gene (*ltpgpA*) and the yeast cadmium factor gene (*ycf1*). In addition to alkaloid cytotoxic drugs, MRP1 is resistant to methotrexate (MTX), ZD1694 and GW1843 (*58,59*). The topoisomerase I inhibitors, camptothecin derivative, CPT-11 (irinotecan), and its active metabolite, SN-38 in unconjugated and conjugated forms are also actively effluxed out of cells by MRP1 (*60*). MRP1 confers resistance to doxorubicin, vincristine, etoposide, and mitoxantrone (*61,62*). MRP1 substrates also include conjugates of thiotepa, cyclophosphamide, chlorambucil, and melphalan (*61,63,64*). The resistance capability of MRP1 to melphalan can be increased by co-upregulation of glutathione *S*-transferases or the GSH biosynthetic enzyme, γ-glutamylcysteine synthetase (*63,64*).

MRP1 also confers resistance to arsenic in association with GSH (*56*). The ability of MRP1 to cause arsenite resistance in transfected or selected cellsand the overexpression of MRP1 in cells selected for arsenite (*56*) has raised the question of whether MRP1 might be responsible for the arsenite resistance of patients treated with arsenite for acute promyelocytic leukemia. However, that the *Mrp1*$^{-/-}$ mouse is not hypersensitive to arsenite (*65*), which suggests that MRP1 is not a critical factor in the cellular defense against arsenite. This could be due to the rapid excretion of the complexes of arsenite and methylarsenite with GSH into bile (*66*).

MRP1 transports the protease inhibitors, ritonavir and saquinavir (*67-69*), the antiandrogen drug flutamide and its metabolite hydroxyflutamide (*70*), and the GSH conjugates of ethacrynic acid (a diuretic) (*71*). In addition, the radiopharmaceuticals ^{99m}Tc-Sestamibi, ^{99m}Tc-Tetrofosmin, and the gadolinium chelate B22956/1 are substrates of MRP1 (*72-74*). Such compounds are used in clinical functional imaging studies and recently they may be used for *in vivo* imaging of hepatobiliary transport function.

A number of chemical toxicants and their metabolites are known to be the substrates for MRP1. Aflatoxin B1 and several *S* and *R* GSH conjugate stereoisomers of aflatoxin B1 (*75*), the GSH conjugates of herbicide metolachlor (*76*), and the GSH conjugates of the model toxicants 1-chloro-2,4-dinitrobenzene (*77*) and 4-nitroquinoline 1-oxide (*78*) have been identified as MRP1 substrates. However, a recent study indicated that carcinogen aflatoxin B1 induced a similar number of lung and liver tumors in both *mrp1*-null and wide type mice (*79*). This may be due to the redundancy

of transmembrane export pumps, other pumps may effectively vicariate for MRP1-mediated transport of aflatoxin B1 and its glutathione conjugates. In addition, the 3β-*O*-glucuronide conjugate of the tobacco metabolite 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL) is also a substrate of MRP1 (*53*). Notably, the NNAL-*O*-glucuronide transport by MRP1 requires physiological concentrations of GSH (*53*). NNAL is a lung cancer inducer.

MRP1 and murine Mrp1 are normally located in intracellular vesicles of undefined nature and in the basolateral membrane of epithelial membranes. Hence, MRP1 secretes drugs into the body, rather than moving them out of the body as PgP or MRP2 do. This makes MRP1 a system of cellular defense rather than one of total organism defense like Mdr1 PgP and MRP2, which eliminate drugs from the body. The importance of this cellular function is highlighted by the fact that mice lacking Mrp1 are hypersensitive to etoposide (*65,80*), whereas an increased sensitivity to vincristine is uncovered in the TKO mice (triple knockout mice in which the disrupted *Mrp1* alleles are combined with disruptions of the two drug-transporting PgP (ABCB1) genes, *Mdr1a* and *Mdr1b*) (*80*). In mice, loss of Mrp1 is associated only with increased sensitivity to epipodophyllotoxins (*e.g.* etoposide) and *Vinca* alkaloids (*e.g.* vincristine), the drugs also most affected by the absence of Mrp1 in *Mrp1*$^{-/-}$ embryonic stem cells (*65*). Knockout mice without *mrp1* have a decreased response to inflammatory stimuli, increased levels of GSH, and increased sensitivity to etoposide but are otherwise healthy and fertile (*41,65*).

A variety of inhibitors of MRP1 have been identified, but their specificity as yes to be determined. Some general inhibitors of organic anion transport including probenecid, sulfinpyrazone and indomethacin are able to inhibit MRP1 (*81-83*). The inhibitors of PgP such as verapamil, quercetin, genistein and cyclosporine can also suppress the transport activity of MRP1 (*84-88*). Other PgP and MRP1 dual inhibitors include the dihydropyridine PAK-104P (*89*), the polyhydroxylated sterol acetate agosterol A (*90*), steroid analogs (*91,92*), and imidazothiazole derivatives (*93*). The MRP1 inhibiting bioflavonoids, such as genistein, quercetin, biochanin A, and kaempferol, can also decrease the intracellular GSH levels (*85-88*). The non-nucleoside reverse transcriptase inhibitors (delavirdine, efavirenz, and nevirapine), nucleoside reverse transcriptase inhibitors (abacavir, emtricitabine, and lamivudine), and tenofovir as a nonnucleotide reverse transcriptase inhibitor also inhibited MRP1 *in vitro* (*94*).

There are some inhibitors specific to MRP family members. For example, the LTD_4 receptor antagonist, MK571, is a GSH conjugate inhibiting both MRP1 and MRP2 (*95*). Different to MK571 in structure, the peptide leukotriene receptor antagonist ONO-1078, has also been demonstrated to reduce LTC_4-efflux in lung tumor cells by blocking MRP1 function (*96*). The sulphonylurea, glibenclamide also shows inhibitory activity to both MRP1 and MRP2 (*97*). In addition, several highly specific ad potent MRP1 inhibitors have been identified. These include tricyclic isoxazole derivatives such as LY475776 and LY402913 (*98-100*). It has been reported that some antisense oligonucleotides are also able to inhibit MRP1 activity by reducing MRP1 mRNA levels and the protein synthesis (*101-103*). For instance, some antisense oligonucleotides reduce the expression level of the MRP1 protein by 46% and its mRNA level by 76% (*103*). ISIS 7597, an antisense oligonucleotide, is able to quickly decrease intracellular MRP1 mRNA levels by up to 90% at a low concentration (0.5 μM) (*101*).

MRP2 (ABCC2) is also known as the canalicular multispecific organic anion transporter (cMOAT). The amino acids of MRP2 have 49% identity with MRP1 (*104*). Human *MRP2* maps to chromosome 10q23-24 and consists of 32 exons spanning 65 kb (*105*). The location of MRP2 is unique, as it is present on the apical plasma membranes of polarized cells such as hepatocytes, pneumocytes, kidney proximal tubules, and specialized cells in the intestine and brain (*106,107*), while other MRPs are all located on basolateral membrane of polarized cells. Based on its localization and substrate specificity, it is proposed that the primary physiological function of MRP2 is to export amphiphilic organic anions and xenobiotics into bile and into the lumen of excretory organs (*108*).

Like MRP1, MRP2 transfected cells are resistant to etoposide, vinca alkaloids, anthracyclines, camptothecins, CPT-11 and MTX (*59,109-111*). The substrates of MPR1 and MRP2 have similarity with regard to the transport of GSH and glucuronate, and sulfate conjugates, but there are some important differences. The affinity of MRP2 to GSH conjugates is less than that of MRP1 (*112,113*). For instance, the affinity to MRP2 for both LTC_4 and *N*-ethylmaleimide glutathione is found to be significantly lower than that of MRP1 (*83*), whereas bilirubin mono- and bis-glucuronides have higher affinity for MRP2 (*106,114*). MRP2 is distinct from MRP1 with the ability to confer resistance to cisplatin (*109-111*), probably in the presence of GSH (*48*). Cisplatin resistance in MRP2-overexpressing cells is thus abrogated by MRP2 antisense cDNA. GSH itself appears to be a relatively low affinity substrate for MRP2 (*115*), but the co-transport of GSH with MRP2 substrate is similar to that observed for MRP1 (*113,116*).

MRP2 transports an array of conjugated endogenous metabolites. In addition to LTC_4, GSH, GSSG, and bilirubin conjugates, MRP2 is able to transport LTD_4, LTE_4, and the glucuronide conjugates of estrodiol and triiodo-L-thyronine (*112*). The substrates of MRP2 also include the glucuronide conjugates of grepafloxacin, diclofenac and acetaminophen (*112,117,118*). Moreover, sulfated MRP2 substrates include taurolithocholate sulfate and taurochenodeoxycholate sulfate, but not

estrone 3-sulfate (*119,120*).

MRP2 also transport ampicillin, ceftriaxone, pravastatin, temocaprilat, grepafloxacin and BQ-123 (*119,121*). Olmesartan, a novel angiotensin II blocker, is a substrate of MRP2 (*122*). A previous study reported that the biliary excretion of olmesartan is mediated by Mrp2 based on low biliary excretion in Eisai hyperbilirubinemic rats (EHBR), which are inherited mrp2-deficient rats, compared with Sprague-Dawley rats (*123*). Moreover, the HIV protease inhibitors saquinavir, lopinavir, ritonavir and indinavir are MRP2 substrates (*124,125*). Similar to MRP1, MRP2 can transport ^{99m}Tc-labeled compounds used in functional imaging studies (*126*).

Interestingly, MRP2 shows its ability to transport certain carcinogens and other toxicants as conjugates or as unconjugated organic anions. For example, MRP2 can transport the tobacco carcinogen NNAL, and in contrast to MRP1, GSH is not needed (*53*). MRP2 is also capable of transporting the GSH conjugate of (+)-anti-benzo[a]pyrene-7,8-diol-9,10-epoxide, the active metabolite of benzo[a]pyrene (*127*). Other toxicants as substrates of MRP2 include arsenite, cadmium and α-naphthylisothiocyanate with the need of GSH (*128,129*). This suggests a role of MRP2 in chemoprotection in the body.

Many inhibitors of MRP2 have been established, and most of which do not have high selectivity to MRP2. For instance, MK571 can also inhibit MRP1 and MRP3. The organic anions have different inhibitory effects on MRP2. For example, probenecid and furosemide inhibit, whereas under certain conditions, sulfinpyrazone, penicillin G, and indomethacin considerably stimulated MRP2 transport activity (*83*). However, all these compounds inhibit MRP1-ATPase capability. MRP1 may be a more potent transporter of GSH conjugates and free GSH than MRP2, but several anions are preferred substrates for MRP2. This may indicate different modulation selectivity on MRP1 or MRP2 in drug resistant cancer cells (*83*). The MPR2-mediated transport of known substrate $E_2$17βG can be blocked by bile acids and certain amphipathic anions (*130,131*). The antisense cDNA expression is also used to block the drug resistance capability of MRP2 (*132*). The non-nucleoside reverse transcriptase inhibitors (delavirdine, efavirenz, and nevirapine), nucleoside reverse transcriptase inhibitors (abacavir, emtricitabine, and lamivudine), and tenofovir as a nonnucleotide reverse transcriptase inhibitor also inhibited MRP2 *in vitro* (*94*).

Among the MRP family, MRP3 has the highest amino acid sequence resemblance (58%) with MRP1 (*133*). Less is known about this protein than either MRP1 or MRP2. Although most closely related to MRP1 and MRP2, MRP3 has its own particular pattern of tissue localisation and substrate specificity. MRP3 mRNA is mainly detected in small intestine, pancreas, colon, placenta, and adrenal gland, while lower levels are found in liver, brain, kidney and prostate (*134-136*). MRP3 is mainly localized in the basolateral membrane of polarized cells such as cholangiocytes, hepatocytes an enterocytes (*130*).

MRP3 confers resistance to a much narrower spectrum of anticancer drugs compared to MRP1 and MRP2, and the drugs are limited to vincristine, methotrexate, epipodophyllotins (etoposide and teniposide) (*137,138*). MRP3-mediated transport of etoposide is inhibited by some organic anion transport inhibitors, but is not influenced by the reduction of intracellular GSH level. MRP3 is also involved in the transport of $E_2$17βG, LTC_4, dinitrophenyl *S*-glutathione, acetaminophen glucuronide, but not GSH and etoposide glucuronide (*139,140*). Both etoposide and MTX can block the MRP3-mediated transport of $E_2$17βG (*141*). Unlike MRP1 and MRP2, MRP3 has a higher affinity to glucuronate conjugates than to GSH conjugates (*142*). Furthermore, the resistance capacity of MRP3 to etoposide and vincristine is much lower than that of MRP1. However, MRP3 shows poor resistance to some natural product drugs, such as anthracyclines and Taxol (*138*). MRP3 is present in cancer cell lines from many tissues, but initial studies on MRP3 in a panel of drug-resistant cancer cell lines did not turn up any association between MRP3 levels and drug resistance (*143*). However, there was a strong correlation between MRP3 and doxorubicin resistance in lung cancer lines (*144*).

In contrast to MRP1 and MRP2, MRP3 has a greater capacity to transport glucuronate conjugates than GSH conjugates, and it can not increase GSH efflux in transfected cells (*145*). MRP3 also transports monovalent bile salts such as cholate, glycocholate and taurocholate which are not substrates for MRP1 and MRP2 (*138,146*). Conversely, the conjugated cholate 3-*O*-glucronide, taurochenodeoxycholate 3-sulfae and taurolithocholate-3-sulfte are substrates for all three MRP proteins (*139*). Thus, MRP3 may have a role in entero-hepatic circulation of bile salts and it is considered to function as a backup detoxifying pathway for hepatocytes when normal canalicular route is damaged by cholestatic diseases and the function of MRP1 and MRP2 is impaired (*147-149*). The non-nucleoside reverse transcriptase inhibitors (delavirdine, efavirenz, and nevirapine), nucleoside reverse transcriptase inhibitors (emtricitabine, and lamivudine), and tenofovir as a nonnucleotide reverse transcriptase inhibitor also inhibited MRP3 *in vitro* (*94*).

MRP4 (ABCC4) has particular tissue expression profile, drug resistance selectivity, and substrate and inhibitor specificity, in comparison with other MRPs. Although MRP4 mRNA is present in most organs, MRP4 protein is mainly detected in the kidneys (*134*). MRP4 is a lipophilic anion pump capable of transporting some physiological and endogenous compounds. These include cyclic adenosine monophosphate (cAMP) and cyclic guanosine monophosphate (cGMP), GSH (*150*),

and folate (*151-153*). MRP4 is also able to mediate the uptake of PGE_1 and PGE_2, while MRP1, MRP2, MRP3, and MPR5 can not transport PGE_1 and PGE_2 (*154,155*).

MRP4 is able to transport several endogenous organic anions and steroid conjugates, including $E_2$17βG (*35,36,139*), and dehydroepiandrosterone-3-sulfate (DHEAS) which is the major circulating steroid made in the adrenal gland in humans (*156*). The affinity of MRP4 for $E_2$17βG is similar to that of MRP3, while lower than that of MRP1 and MRP2 (*35,36,139*). No transport of DHEAS by MRP2 or MRP3 is found (*156*). MRP4 mediates ATP-dependent co-transport of GSH or *S*-methyl-glutathione together with cholyltaurine, cholylglycine, or cholate (*157*). A recent study has identified conjugated bile acids, especially sulfated derivatives, as substrates of MRP4 (*156*). Bile acids, like the steroid $E_2$17βG, contain a cholesterol backbone structure and may thus represent physiological substrates of MRP4. GSH plays an important role in the function of MRP4, as MRP4 transports many of its substrates in a GSH-dependent manner and depletion of intracellular GSH by the GSH synthesis inhibitor, DL-buthionine-(*S*,*R*)-sulphoximine, blocks the MRP4-mediated export of cAMP and abolishes resistance to nucleoside analogues (*150*). MRP4 participates in the hepatic basolateral excretion of sulfate conjugates (*158*).

A variety of nucleoside (purine and pyrimidine) analogues are found to be substrates for MRP4. These include ganciclovir (*159*), azidiothymidine monophosphate (*160*), 9-(2-phosphonylmethoxyethy l)adenine (PMEA) (*160,161*), bis(pivaloxymethyl)-9-(2-phosphonylmethoxyethyl)adenine, a lipophilic ester prodrug) (*162*), 6-mercaptopurine, and 6-thioguanine (*151*). ATP-dependent uptake of the acyclic nucleotide phosphonates, adefovir and tenofovir but not cidofovir, was observed only in the membrane vesicles expressing MRP4 (*163*). The kidney accumulation of adefovir and tenofovir was significantly greater in Mrp4 knockout mice (130 *versus* 66 and 191 *vs* 87 pmol/g tissue, respectively); thus, the renal luminal efflux clearance was estimated to be 37 and 46%, respectively, of the control (*163*). There was no change in the kinetic parameters of cidofovir in Mrp4 knockout mice. There was no difference in the fraction of mono- and diphosphorylated forms of adefovir in the kidney between wild-type and Mrp4 knockout mice (*163*). These findings indicate that MRP4 is involved in the renal luminal efflux of both adefovir and tenofovir, but it makes only a limited contribution to the urinary excretion of cidofovir. MRP4 is also an efflux pump for urate, the purine end metabolite (*164*) and thioxanthosine monophosphate and thioinosine monophosphate (both thiopurine metabolites) (*165*). Moreover, MRP4 transports the anticancer agents topotecan (*166*), leucovorin (*152*), and MTX (*137,152,161*). Topotecan is a semi-synthetic, water-soluble derivative of camptothecin, a cytotoxic plant alkaloid isolated from the Chinese tree *Camptotheca acuminata* (*167*). It is used as a second-line treatment for patients with ovarian carcinoma. Moreover, MRP4 can mediate the efflux of the glutathione conjugate of monochlorobimane, a bimane that forms fluorescent adduct with thiols (*168*).

A variety of inhibitors for MRP4 have been identified. Like MRP1 and MRP2, MRP4 is also inhibited by the leukotriene antagonist MK571 (*151,153*). The cellular efflux of cGMP by both MRP4 and MRP5 is inhibited by PGA1 and PGE1, the steroid progesterone and the anticancer drug estramustine (a combination of estrogen and mechlorethamine) (*169*). PGA1 inhibited the ATP-dependent efflux of MTX, another MRP4 substrate (*152,170*). PGF1α, PGF2α, PGA1, and thromboxane B2 are high-affinity inhibitors (therefore presumably substrates) of MRP4-mediated transport of PGE1 and PGE2 (*171*). The MRP4-mediated transport of PGE1 and PGE2 is also inhibited by rofecoxib and celecoxib (both COX-2-specific inhibitors), and diclofenac (*171*). Sulfinpyrazone is a potent inhibitor (IC_{50} = 420 μM) of PMEA efflux in MRP4-overexpressing HEK293 cells (*171*). MTX can inhibit the MRP4-mediated transport of $E_2$17βG (*151*). Glucuronide and glutathione conjugates can also inhibit MRP4-mediated transport of MTX (*152,153*). The MRP4-mediated transport of $E_2$17βG is blocked in the presence of estradiol 3,17-disulphate, taurolithocholate 3-sulphate (*156*), or topotecan (*166*). The MRP4-mediated transport of bimane-glutathione is totally inhibited in the presence of carbonylcyanide *m*-chlorophenylhydrasone (an uncoupler of oxidative phosphorylation) and significant inhibition is also observed with known inhibitors of MRP transporters including benzbromarone, verapamil, indomethacin, MTX, and 6-TG (*168*). Such transport is also inhibited by 1-chloro-2,4-dinitrobenzene (CDNB) which is metabolized to the glutathione conjugate after entry into cells.

MRP4 may be regulated at transcriptional, translational and posttranslational level. Its expression is substantially increased in livers of mice with disruption of the farnesyl/bile acid nuclear receptor, which have increased levels of serum and hepatocellular bile acids, and MRP4 can be further upregulated by cholic acid feeding (*172*). The constitutively active nuclear receptor (CAR) is required to coordinately upregulate hepatic expression of MRP4 and an enzyme known to sulfate hydroxy-bile acids and steroids (Sult2a1) (*173*). CAR activators increased MRP4 and Sult2a1 expression in primary human hepatocytes and HepG2, a human liver cell line. Sult2a1 was down-regulated in MRP4-null mice, further indicating an inter-relation between *MRP4* and *Sult2a1* gene expression. Based on the hydrophilic nature of sulfated bile acids and MRP4's capability to transport sulfated steroids, these findings suggest that MRP4 and Sult2a1 participate in an integrated pathway mediating elimination of sulfated steroid and bile-acid metabolites from the liver. In addition, a recent

study in infected human macrophages indicates that azidiothymidine treatment induces MRP4 mRNA (*174*).

Analysis of tissue RNA suggests that *MRP5* is ubiquitously expressed. The highest levels are found in skeletal muscle and brain (*143*). In comparison with MRP1-3, MRP5 (ABCC5) has its particular drug resistance selectivity and shows no resistance to natural anticancer compounds or MTX. MRP5 and MRP4 share only 36% amino acids identity, and their substrate specificity is similar. Both MRP4 and MRP5 are able to mediate the Mg^{++}/ATP-dependent transport of cGMP and cAMP. MRP4 has a higher affinity for cAMP than that of MRP5, while MRP5 has a higher affinity for cGMP that of MRP4 (*151*). Like MRP4, MRP5 is capable to transporting purine derivatives including PMEA and 6-mercaptopurine (*175,176*). However, MRP4 is also able to transport some substrates of MRP1-3, such as $E_2$17βG and MTX (*137*). MRP5 is able to transport *S*-(2,4-dinitrophenyl)glutathione which is inhibited by typical organic anion transport inhibitors, including sulfinpyrazone and benzbromarone (*175*). However, most glutathione and glucuronate conjugates are not substrates of MRP5. Notably, MRP5 shows resistance to heavy metals including cadmium chloride and potassium antimonyl tartrate (*176*). MRP5 can be modulated by general organic anion transport inhibitors, including probenecid, sulfinpyrazone, benzbromarone, and MK571 (*171*). Like MRP4, there are no specific inhibitors of MRP5.

The physiological functions and possible role in drug resistance of MRP4 and 5 remain to be defined. Obviously, the discovery that these pumps can transport cyclic nucleotides, notably cGMP, has raised the question of whether MRP4/5 can affect the signal transduction role of cGMP by removing it from the cell, which would supplement the degradation by phosphodiesterases. There is also evidence for an extracellular signaling role for cGMP in kidney and several other tissues, and MRP4/5 might be involved. No human disease has been associated with alterations in MRP5, and the Mrp5 KO mouse, generated by Wijnholds *et al*. (*175*), has no obvious phenotype. It is possible, however, that the overlapping substrate specificities of MRP5 and MRP4 (and possibly MRP8 and 9) may hide the physiological function of Mrp5, *e.g*., in cyclic nucleotide transport, and that the breeding of mice lacking all these transporters may lead to an understanding of the physiological function of each of them.

Human MRP6 is most closely related to MRP1 and MRP2 with 45% and 43% amino acid identity, respectively. The highest levels of *MRP6* mRNA and protein expression are detected in kidney and liver while low levels are found in most other tissues such as skin and retina (*177-179*). MRP6 is located on the basolateral membranes in hepatocytes and kidney proximal tubules (*180*). Overexpression of MRP6 does lead to weak resistance to chemotherapeutic drugs (*181*). Rat Mrp6 transported the cyclic cyclopentapeptide endothelin-1 receptor antagonist BQ123, although endothelin-1 itself is not a substrate of Mrp6 (*182*). However, rat Mrp6 did not transport glucuronide, sulfate and GSH conjugates, hydrophobic drugs, PGs or aminophospholipids (*182*). More recently, MRP6 was found to transport glutathione conjugates, such as LTC_4, *N*-ethylmaleimide, *S*-glutathione and dinitrophenol glutathione, while $E_2$17βG appears a poor MRP6 substrate (*181,183*). Effective inhibitors of MRP1 and MRP2, including indomethacin, probenecid, and benzbromarone, can block the MRP6-mediated transport (*183*). MRP6 also exhibited low-level resistant activity to a variety of natural product anticancer drugs, such as etoposide, teniposide, doxorubicin, cisplatin, daunorubicin and dactinomycin (*181*). These findings suggest that MRP6 may transport conjugated organic anions and probably confers resistance to anticancer drugs to a less effective extent than MRP1-3.

MRP7 (ABCC10) has the lowest amino acid sequence identity (33-36%) with other MRP family members (*18*). Although MRP7 mRNA can be detected in most tissues, but the expression levels are usually very low (*18*). MRP7 is able to transport $E_2$17βG with a high K_m (58 μM) (*184*). This suggests that MRP7 may be a lipophilic anion transporter. In contrast, MRP7 did not transport other typical MRP substrates, such as cyclic nucleotides, MTX, or bile acids (*184,185*). Interestingly, MRP7 is as closely related to the SUR K_i channel regulators, but the functional implication is yet to be determined.

MRP8 (ABCC11) has 40% amino acids identity with MRP5, and has been characterized as an amphipathic anion transporter. MRP8 is mainly present in normal breast and testis, while little is present in liver, brain, and placenta (*19*). With the ability to efflux cAMP and cGMP, MRP8 confers resistance to purine and pyrimidine nucleotide derivatives, including anticancer fluoropyrimidines, and several antiviral agents. Similar to the case for other MRPs that possess only two membrane spanning domains (MRP4 and MRP5), MRP8 is a cyclic nucleotide efflux pump that is able to confer resistance to nucleoside-based agents, such as PMEA and 5-FU (*186*). In contrast, little resistance is found for some natural product anticancer drugs (*187*). Recently, MRP8 is found to transport a variety of physiological and synthetic lipophilic anions, including the LTC_4, steroid sulfates such as dehydroepiandrosterone (DHEAS) and estrone 3-sulfate, $E_2$17βG, leukotriene C_4 and dinitrophenyl-*S*-glutathione, the monoanionic bile acids glycocholate and taurocholate, and MTX (*188-191*).

Both *MRP8* and *MRP9* genes are identified using a functional genomic approach and bioinformatics tools. Both MRP8 and MRP9 (ABCC12) have the highest degree of similarity with MRP5. One major difference between MRP8 and MRP9 is that MRP9 has only one ATP-binding domain but two transmembrane domains

each with four membrane-spanning regions. The *MRP9* gene is unusual because it encodes two transcripts of different sizes (*192*). The larger 4.5-kb RNA is found in breast cancer, normal breast, and testis and encodes an MRP-like protein that lacks transmembrane domains 3, 4, 11, and 12 and the second nucleotide-binding domain. The smaller 1.3-kb RNA is detected in brain, skeletal muscle, and ovary and seems to encode the second nucleotide-binding domain. There is a lack of information on the substrate specificity of MRP9. It is speculated that MRP9 may have a different function from other family members. Because both MRP8 and MRP9 are membrane proteins with very restricted expression in essential tissues (*21*), they may represent potential molecular targets for targeted therapy with antibodies, antibody conjugates, and immunotoxins.

Various MRPs show considerable differences in their tissue distribution, substrate specificities, and proposed physiological and pharmacological functions. The tissue distribution, substrates and inhibitors of MRPs are listed in Table 1. MRPs are capable of transporting a structurally diverse array of endo- and xenobiotics including many therapeutic drugs and their metabolites across cell membranes. They play an important role in the absorption, disposition and elimination of many therapeutic agents in the body.

4. Induction of MRPs

Regulation of ABC transporter gene expression involves participation of numerous nuclear receptors (*193-195*). Nuclear receptors constitute a family of transcription factors that act as heterodimers, which bind to promoter elements and induce gene expression. Transporter genes are regulated at several levels, including membrane retrieval and reinsertion, translation, and transcription. Nuclear receptors relevant for the expression of ABC transporters are liver X receptor (LXR), farnesoid receptor (FXR), pregnane X receptor (PXR), and peroxisome proliferator-activated receptors α and γ (PPARα and PPARγ) (*4*). The induction of *CYP3A4* and *CYP2B6* genes by numerous xenobiotics is well known to be mediated through activation of PXR (*196*). PXR is activated by a diverse number of compounds, including rifampicin, phenobarbital, and mifepristone in humans. PXR mediates the expression of rodent Oatp1a4 (*194*), Oatp2 (*197,198*), human MDR1 (*199*), mouse MRP1 (*200*), Mrp2 (*200*) and Mrp3 (*201*). Furthermore, CAR activation induces Mrp2-7 mRNA in mouse liver (*202*) and is involved in the regulation of Mrp4 and sulfotransferase 2A1 (*173*). The PPARα agonist clofibrate induces gene and protein expression of Mrp3 and Mrp4 efflux transporters in a PPARα-dependent manner while having little effect on mRNA expression of Ntcp, Oatp1a1, Oatp1a4, and Oatp1b2 uptake transporters in mouse liver (*203*).

In primary cultures of human hepatocytes, MRP1 was increased by rifampin (*204*). In mouse liver, carbon tetrachloride induced Mrp1 (*205*). MRP1 is up-regulated when exposed to rifampin (*206,207*) or mitoxantrone in tumor cells (*208*). The expression of MRP1 in human colorectal cancer cell lines was induced by sulindac (*209*).

The promoter regions of the human *MRP2* and the rat *Mrp2* gene contain a number of putative consensus binding sites for AP1, SP1, HNF1, and HNF3β (*210*). The -431 to -258 region also contains important elements that control expression in HepG2 cells, particularly the CCAAT-enhancer binding protein β. AhR ligands (2,3,7,8-tetrachlorodibenzo-*p*-dioxin, polychlorinated biphenyl 126, and β-naphthoflavone), the CAR activator 1,4-bis[2-(3,5-dichloropyridyloxy)]benzene, and nuclear factor-E2-related factor 2 (Nrf2) activators (butylated hydroxyanisole, oltipraz, and ethoxyquin) increased Mrp2 expression in mouse liver, suggesting that AhR, CAR, and Nrf2 may be important for modulating Mrp2 expression by chemicals (*202*). Induction of rat Mrp2 has been observed with numerous chemicals, such as pregnenolone-16α-carbonitrile, spironolactone, and dexamethasone (all PXR ligands), phenobarbital (CAR ligand), and oltipraz (Nrf2 activator) (*211,212*). Similar induction of Mrp2 with indole-3-carbinol and β-naphthoflavone, both AhR ligands, has also been observed in rat liver (*213*). Ligands for FXR, PXR, and CAR all induced Mrp2 mRNA in primary cultures of rat hepatocytes and characterized a putative ER-8 at -401 to -376 of the rat Mrp2 promoter that bound the corresponding FXR/RXR, PXR/RXR, and CAR/RXR heterodimers (*214*). Treatment with the chemical carcinogen 2-acetylaminofluorene, cisplatin, and the protein-synthesis inhibitor cycloheximide increased expression of Mrp2 in rat liver (*215*). *trans*-Stilbene oxide also induced rat Mrp2 expression *via* CAR-independent manner (*216*).

The inducibility of *Mrp2* gene expression in primate liver was investigated in rhesus monkeys treated with tamoxifen or rifampin (*217*). Both tamoxifen and rifampin strongly induced Mrp2 mRNA in two male and two female rhesus; tamoxifen induced Mrp2 protein in both male and female rhesus, whereas rifampin showed some inducing effect in a female but was inactive in a male monkey. Carotenoids and retinol also induced MRP2 through PXR activation (*218*). Human MRP2 is similarly up-regulated by the PXR activators rifampicin and tamoxifen, which differ from known rodent ligands for PXR (*219*). Similarly, MRP2 is induced by phenobarbital (*220*) and by *tert*-butyl hydroquinone in HepG2 cells (*200*), which suggests that CAR and Nrf2, respectively, may regulate expression of the human *MRP2* gene. These results suggest that the gene for *Mrp2* may be similarly up-regulated by PXR agonists in human and rat, but mouse Mrp2 may not be as sensitive to PXR ligands. In clinical studies, expression of MRP2 mRNA and protein was decreased in patients with obstructive cholestasis who were poorly drained by percutaneous

Table 1. Tissue distribution, substrates and inhibitors of MRPs

Name	Symbol	Tissue location	Expression levels	Major drug substrates	Physiologic substrates	Inhibitors
MRP1	ABCC1	All major tissues	Differ in various organs and cell lines	Doxorubicin, vincristine, etoposide, MTX, camptothecin, CPT-11, SN-38, cyclophosphamide, conjugates	Glutathione, LTC4, $E_2$17βG, sulfated bile acids, bilirubin, PGA GSH conjugate, GSH, GSSG	Probenecid, sulfinpyrazone, indomethacin, verapamil, quercetin, genistein, cyclosporine, PAK-104P, steroid analogs, MK571, ONO-1078, sulphonylurea, glibenclamide
MRP2	ABCC2, cMOAT	Liver, kidney, intestine, brain		Conjugates, cisplatin, etoposide, vinca alkaloids, anthracyclines, Camptothecins, MTX, lopinavir, olmesartan	LTC4, GSH, GSSG, bilirubin conjugates, LTD4, LTE4	MK571, furosemide
MRP3	ABCC3	Small intestine, pancreas, colon, placenta, adrenal gland	Low level in liver, brain, kidney and prostate	Etoposide, teniposide, dinitrophenyl *S*-glutathione, acetaminophen glucuronide, vincristine, MTX	LTC4, $E_2$17βG, cholate, glycocholate, taurocholate	Etoposide, MTX
MRP4	ABCC4	Kidneys	Low levels in other tissues	MTX, 6-thioguanine, PMEA, 6-mercaptopurine, topotecan	cGMP, cAMP, DHEAS, $E_2$17βG, PGE_1, PGE_2	MK571, celecoxib, rofecoxib, diclofenac
MRP5	ABCC5	Most tissues	Low levels	6-Mercaptopurine, 6-thioguanine, PMEA, heavy metals, *S*-(2,4-dinitrophenyl)glutathione	cGMP, cAMP	Probenecid, sulfinpyrazone, benzbromarone, MK571
MRP6	ABCC6	Liver, kidney	Low levels in other tissues	LTC4, *N*-ethylmaleimide *S*-glutathione, dinitrophenol glutathione, etoposide, doxorubicin, cisplatin, daunorubicin	?	Indomethacin, probenecid, benzbromarone
MRP7	ABCC10	Most tissues	Very low levels	?	$E_2$17βG	?
MRP8	ABCC11	Normal breast, testis	Low levels in liver, brain, and placenta	5-FU, ddC, PMEA, MTX, bile acids	cGMP, cAMP, LTC4, DHEAS,	?
MRP9	ABCC12	Breast cancer, normal breast, testis, brain, skeletal muscle, ovary	Low levels	?	?	?

transhepatic biliary drainage (*221*). In another clinical study, rifampin treatment of normal human subjects increased MRP2 mRNA and protein in the duodenum (*222*). Additionally, induction of chronic renal failure in rats increased Mrp2 mRNA and protein levels in both the kidney and the liver (*223*). This may represent a compensatory mechanism during renal failure, although the human response has not yet been documented.

The expression of MRP3 in rat and human liver is low under normal conditions but is induced during cholestasis and in the absence of MRP2 or bile salt export pump (BSEP) (*172*,*224*). Bile acids, in particular lithocholic acid, have been demonstrated to activate PXR likely as a mechanism to control their production and metabolism to prevent their accumulation to toxic levels (*225*). In rats, mice, and humans, Mrp3 has been shown to be regulated by phenobarbital, diallyl sulfide, and polychlorinated biphenyl 99 (*226*), compounds that induce Cyp2B1/2 and are known or hypothesized CAR activators. *trans*-Stilbene oxide also induced rat Mrp3 expression *via* CAR-independent manner (*216*). Similar to Mrp2, Mrp3 is highly up-regulated by oltipraz (*202*), suggesting that Nrf2 might be an important transcription factor that regulates Mrp3 (*226*). In humans, induction by β-naphthoflavone and rifampicin suggests that MRP3 might be regulated *via* AhR or PXR, respectively (*220*). Using a large collection of human liver tissues, it was found that omeprazole was an inducer of MRP3 expression, probably through a AhR-dependent pathway (*227*). This effect could be reproduced with HepG2 hepatoma cells, which showed a concentration-dependent induction of MRP3 expression by omeprazole. Overall, Mrp3 seems to be regulated similarly in rats, mice, and humans, with potential transcriptional regulation by AhR, PXR, CAR, PPARα, and Nrf2.

The CAR activator 1,4-bis[2-(3,5-dichloro-pyridyloxy)]benzene and Nrf2 activators (butylated hydroxyanisole, oltipraz, and ethoxyquin) induced Mrp4 in mouse liver (*202*), indicating potential roles for CAR and Nrf2 in the regulation of mouse Mrp4. In rats, Mrp4 is induced in liver by the Nrf2 activators oltipraz, and ethoxyquin (*228*). *trans*-Stilbene oxide also induced rat Mrp4 expression *via* CAR-independent manner (*216*). Little data exists on induction of MRP4 in humans. However, studies in CAR-null mice have definitively shown that induction of Mrp4 by 1,4-bis[2-(3,5-dichloropyridyloxy)]benzene and phenobarbital is *via* CAR (*173*). Taken together, the most likely means of induction of Mrp4 is by transcriptional activation by CAR and Nrf2.

Few chemicals have been observed to modulate expression of MRP6 in rats or humans. However, AhR, CAR, and Nrf2 activators induced expression of Mrp6 in mouse liver (*202*). A recent study found that the expression of this gene in cells of hepatic origin is significantly upregulated by retinoids, acting as agonists of the retinoid X receptor (RXR) rather than the retinoid A receptor (RAR) (*229*).

One of the patterns of Mrp expression of note is that AhR and Nrf2 activators often induce the same transporter (*i.e.*, Mrp2, 3, 5, and 6). Several genes known to be regulated by Nrf2 are also regulated in a similar manner compared with these Mrps. Rat UDP-glucuronosyltransferase 1A6 is induced by oltipraz, a classical Nrf2 activator, and oltipraz induction of UDP-glucuronosyltransferase 1A6 is dependent on the binding of AhR to the xenobiotic response element (*230*). Furthermore, one of the known target genes of Nrf2 activation, Nqo1, can be induced by the classical AhR ligand 2,3,7,8-tetrachlorodibenzo-*p*-dioxin, and that induction was Nrf2-dependent (*231*). Although the mechanism of this cross-activation is not well defined, MRPs may share a similar pattern of inducibility to the phase I and II enzymes known to be regulated by these two receptors. Thus, it is unclear whether the induction of MRPs by some of the microsomal enzyme inducers is mediated through direct mechanisms (transcription factor binding to its cognate response element) or indirect mechanisms that involve some sort of "cross talk" (activation of multiple receptors by a chemical and/or transcriptional up-regulation of another gene or transcription factor that acts on the gene of interest.

5. MRPs and intestinal absorption of drugs

Many orally administered drugs must overcome several barriers before reaching their target site (*232*). The first major obstacle to cross is the intestinal epithelium. MRP2 and MRP4, together with PgP/MDR1 (ABCB1) and BCRP/MXR (ABCG2), have been shown to localize at the apical/lumenal membrane of enterocytes, and thus are thought to form a barrier to intestinal absorption of substrate drugs (Figure 3) (*232*). Their expression level varies between different segments of the intestine. In general, BCRP/MXR (ABCG2), MRP2 (ABCC2) and PgP/MDR1 (ABCB1) are expressed at high level in the small intestine (*232*), considered by many in the field as the rate limiting barrier to oral drug absorption.

Regarding their role in limiting intestinal absorption, MDR1 is the most thoroughly characterized and well accepted. Although the expression levels of both the MRP2 and MXR are higher in the small intestine than the expression of MDR1, there are much fewer data available on their role in drug absorption (*232*). MRP2 has been shown to limit absorption of a phenylimidazo[4,5-b]pyridine (PhIP) derivative, a food-derived carcinogen, and MXR has been shown to limit absorption of topotecan.

6. MRPs and biliary excretion of drugs

Hepatic transporters are involved in the regulation of bile formation and disposition of xenobiotics. The hepatocyte has a polarized plasma membrane with basolateral

and apical domains, enabling vectorial movement of endogenous and exogenous compounds from blood into bile. Drugs that reach the blood are then passed to the liver, where they are metabolized and subject to biliary excretion, often by MRPs and other important ABC transporters (Figure 4) (*4,232,233*). Canalicular secretion of bile components represents the rate-limiting step in bile formation. Bile acids, glutathione conjugates, and xenobiotics are removed from hepatocytes and concentrated into the bile by canalicular efflux transporters in an ATP-dependent manner.

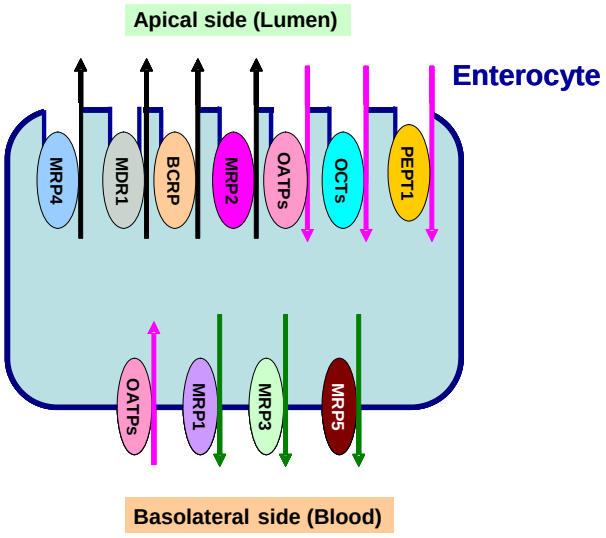

Figure 3. MRP2 and MRP4, together with PgP/MDR1 (ABCB1) and BCRP/MXR (ABCG2), are localize at the apical/lumenal membrane of enterocytes, and thus are thought to form a physical barrier to intestinal absorption of a number of substrate drugs. OATPs, OCTs and PEPT1 are also located at this side. Their expression level varies between different segments of the intestine. In general, BCRP/MXR (ABCG2), MRP2 (ABCC2) and PgP/MDR1 (ABCB1) are expressed at high level in the small intestine, considered by many in the field as the rate limiting barrier to oral drug absorption. MRP1, 3, and 5, and OATPs are expressed at the basolateral membrane of enterocytes.

Four MRP transporters (MRP2, 3, 4, and 6) are expressed to an appreciable extent in liver. In liver, MRP2 is the only MRP localized to the canalicular membrane and participates in excretion of chemicals into bile. Alternatively, MRP3 and MRP4 are localized to the basolateral membrane and efflux chemicals from hepatocytes into blood. MRP6 is thought to be localized to the basolateral membrane as well, but a high-affinity substrate for this transporter has not been identified. The MRPs play an important role in the hepatic elimination of metabolites, and modulation of MRP expression in liver can alter drug disposition.

Both organic cations and anions are taken up into the hepatocyte by groups of transport proteins (OCTs and OATPs respectively) with overlapping specificity. None of the known OATPs import unconjugated bilirubin. Organic anions (including bilirubin and glutathione) are transported across the hepatocyte into bile, usually after being modified by covalent conjugation in the microsomes. These conjugates are secreted into bile by MRP2. After uptake, some compounds may reflux back into the plasma, either by passive diffusion, by MRPs and export by the newly discovered, dimeric organic solute tranporter (OSTα,β) (*234*); these are expressed at the basolateral membrane of the hepatocyte and show considerable overlap of substrate specificity. MRP1 exports both unconjugated and conjugated bilirubins, whereas MRP3 and 4, and OSTα,β best

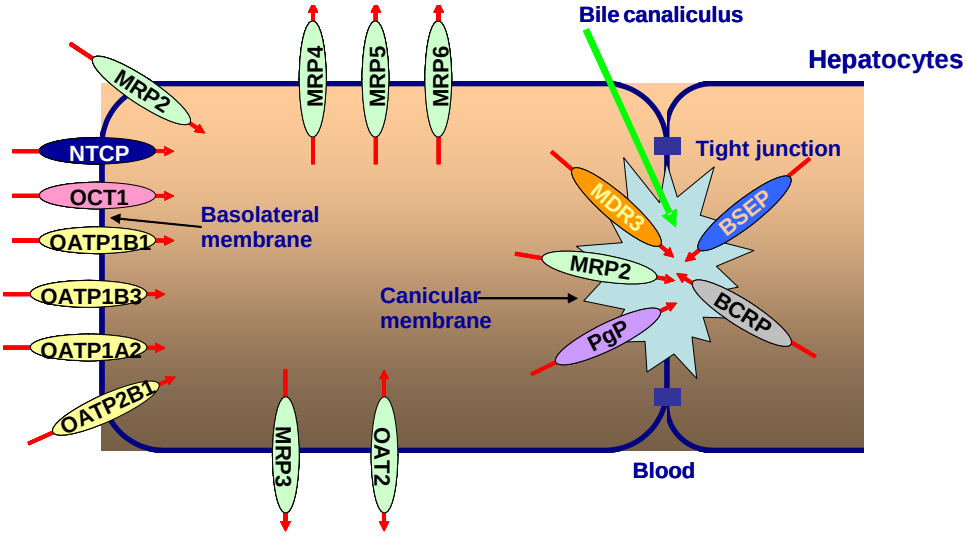

Figure 4. Localization of MRP transporters in hepatocytes. MRP2, localized on the basolateral (sinusoidal) membrane of hepatocytes, plays a critical role in the hepatic excretion of drugs and their metabolites (mainly conjugates). MRP3-6 facilitate the efflux of non-membrane-permeable molecules out of the hepatic cells. Human NTCP (Na^+-taurocholate co-transporting polypeptide) is a Na^+-dependent taurocholate uptake transporter located on the basolateral (sinusoidal) membrane of hepatocytes. NTCP mediates the Na^+-coupled uptake of bile salts from the space of Disse. The conjugated bile salts are then secreted into bile by the canalicular bile salt export pump (BSEP). Phosphatidylcholine (lecithin) is transported to the outer leaflet of the canalicular membrane by the phospholipid flippase, MDR3, from where it is stripped into bile by secreted bile salts. Uptake of organic cations is mediated by a family of organic cation transporters (OCTs). Uptake or organic anions is mediated by families of organic anion transporting polypeptides (OATPs) and organic anion transporters (OATs). Human OATPs, located on the basolateral membrane of hepatocytes, are responsible for the uptake of bile salts, organic anions, hormones, cholates along with their metabolites and conjugates. After conjugation, the organic anions, as well as glutathione, are then secreted into bile by MRP2. A wide variety of amphipathic compounds (including many drugs and organic cations) are exported from the hepatocytes into bile by apical MDR1.

export conjugated bile salts (*234*). All of them have low expression in the normal liver, but are upregulated in cholestasis (*233*,*235*).

Some MRPs (*e.g*. MRP2) play a critical role in the hepatic excretion of drugs and their metabolites (*233*). Decreased MRP function can thus impair hepatic capacity to excrete drugs and their metabolites. For example, altered MRP2 function can change the clearance of many clinically important drugs, including cancer chemotherapeutics (irinotecan, methotrexate, and vinblastine), antibiotics (ampicillin, ceftriaxone, and rifampin), antihyperlipidemics, and angiotensin-converting enzyme inhibitors, as well as many toxins and their conjugates (*236*).

MRP3-6 facilitate the efflux of non-membrane-permeable molecules out of the hepatic cells. Human NTCP (Na^+-taurocholate co-transporting polypeptide) is a Na^+-dependent taurocholate uptake transporter located on the basolateral (sinusoidal) membrane of hepatocytes. The conjugated bile salts are then secreted into bile by the canalicular bile salt export pump (BSEP/ABCB11) (*233*). Phosphatidylcholine (lecithin) is transported to the outer leaflet of the canalicular membrane by the phospholipid flippase (*237*), MDR3, from where it is stripped into bile by secreted bile salts (*238*). Uptake of organic cations is mediated by a family of organic cation transporters (OCTs). Uptake or organic anions is mediated by families of organic anion transporting polypeptides (OATPs) and organic anion transporters (OATs) (*233*,*235*). Human OATPs, located on the basolateral membrane of hepatocytes, are responsible for the uptake of bile salts, organic anions, hormones, cholates along with their metabolites and conjugates (*235*). After conjugation, the organic anions, as well as glutathione, are then secreted into bile by MRP2. A wide variety of amphipathic compounds (including many drugs and organic cations) are exported from the hepatocytes into bile by apical MDR1 (*239*). With regard to the transporters involved in biliary excretion, it is known that PgP (MDR1/ABCB1), MRP2 (ABCC2), the bile salt export protein (BSEP/ABCB11), and BCRP/ABCG2 are predominantly expressed on canalicular membrane (*232*).

7. MRPs and renal drug excretion

PgP/MDR1 (ABCB1), MRP2 (ABCC2), MRP4 (ABCC4) primarily localize to the apical (luminal) membrane of renal epithelial cells, while MRP1 (ABCC1) and MRP6 have been shown to be expressed on the basolateral membrane (Figure 5) (*104*,*240-243*). Substrates of MRP2 and MRP4 have been shown to have altered renal clearance in animals lacking transporter function (*241*). These transporters export compounds from the cytoplasm of renal tubular cells to the urine, therefore, substrates of these transporters are expected to have higher renal elimination than it is expected by glomerular filtration. Tenofovir, an anti-HIV agent, is actively excreted from the proximal tubule cells by MRP2 and MRP4 (*244*). Further studies are needed to understand the detailed role of these transporters in pharamacokinetics.

Additionally, members of the OATP, OCT (OCT1-3) and OAT (OAT1, 3, 4) transporter families have been identified in the basolateral membrane of proximal tubule cells (*241*,*245-247*). OAT3 has shown to be responsible for the renal elimination of pravastatin (*248*). Substrates of OCTs have been shown to have greatly reduced renal clearance and increased plasma concentration in mice lacking OCT1 and OCT2 (*249*). On the other hand, the two peptide transporters PEPT1 and PEPT2 are present on the luminal membrane of proximal tubule cells and were shown to be responsible for the tubular re-absorption of peptide-like drugs such as β-lactam antibiotics across the brush-border membranes (*250*). The reabsorbtion process results in lower renal clearance than it is expected by glomerular filtration. Furthermore, the uptake process might result in increased concentration of drugs in the cytoplasm of proximal tubular cells, leading to toxic effects in the kidney. The nephorotoxic effect of the antibiotic cephaloridine was linked to OAT3 function (*251*,*252*), while OCT2 was identified as the major determinant of the nephrotoxicity of the anti-cancer drug cisplatin (*253*).

MRP2 inhibition by tenofovir may contribute to the known interaction between tenofovir and didanosine. Coadministration of these two antiretroviral drugs leads to an increase of the area under the didanosine concentration-time curve (AUC) by 44 to 60% (*254*). This may occur through tenofovir-induced inhibition of the active uptake of didanosine into the proximal tubule cells by the human organic anion transporter 1 (*255*) or by inhibition of purine nucleoside phosphorylase, an enzyme involved in the degradation of didanosine (*244*,*256*). However, assuming that the MRP2 inhibitor didanosine is also an MRP2 substrate, the increase in didanosine AUC could also be achieved by inhibition of MRP2-mediated efflux in the tubular brush-border membrane or in other tissues. Inhibition of several MRP could also have contributed to the life-threatening toxicity (*e.g*. neutropenia) of the MRP substrate vinblastine in a patient with HIV-associated multicentric Castleman's disease who was maintained on lamivudine, abacavir, and nevirapine (*257*). Another patient with HIV-associated Hodgkin's disease also experienced life-threatening neutropenia when treated with ABVD (doxorubicine, bleomycine, vinblastine, dacarbazine) chemotherapy and lopinavir-ritonavir based antiretroviral therapy (*258*). Vinblastine and lopinavir-ritonavir interaction was managed with lopinavir-ritonavir interruption around chemotherapy administration, with complete remission and immunovirological success after six cycles.

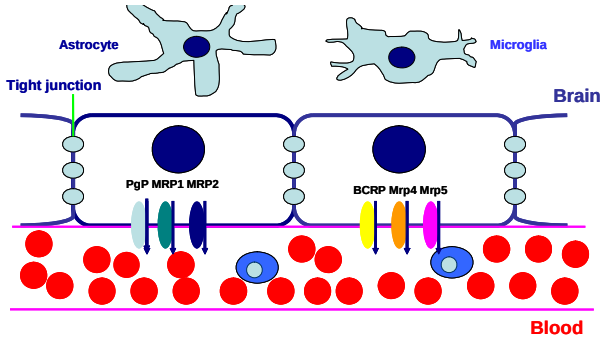

Figure 5. MRP1, MRP2, MRP4, and MRP5 are clearly localized to the luminal (apical) side of brain capillary endothelial cells of the blood-brain barriers. It is well established that the PgP/MDR1 (ABCB1) and BCRP protein localized in the apical/luminal membrane of the brain capillary endothelial cells are a major barrier of brain penetration of drugs. These transporters are also expressed in astrocytes and microglias.

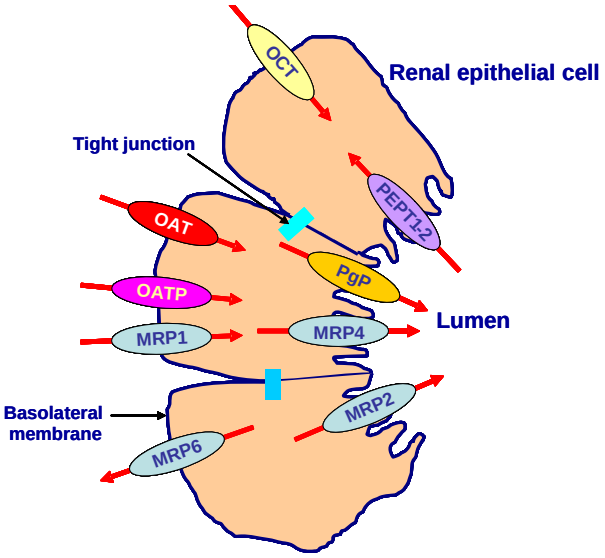

Figure 6. MRP2 (ABCC2) and MRP4 (ABCC4) are primarily localized to the apical (luminal) membrane of renal epithelial cells, while MRP1 (ABCC1) and MRP6 are expressed on the basolateral membrane of proximal tubule cells. PgP/MDR1 (ABCB1) is also located to the apical membrane of renal epithelial cells. Moreover, OATP, OCT (OCT1-3) and OAT (OAT1, 3, and 4) transporters have been identified in the basolateral membrane of proximal tubule cells.

8. MRPs and the blood-brain barrier (BBB)

The BBB is formed by the tight junctions that connect the brain endothelial cells, thus restricting the entry of compounds from the circulating blood to the brain *via* paracellular and transcellular routes (*259-264*). The BBB acts as an anatomical and transporter barrier notably due to the presence of tight junctions and a multitude of ABC transporters such as PgP, BCRP, and MRP1, 2, 4, and 5 (Figure 6) (*4,260,261,264-266*). As such, the BBB contributes to brain homeostasis by protecting the brain from potentially harmful endogenous and exogenous substances (*267*). It is well established that the PgP/MDR1 (ABCB1) and BCRP/MXR(ABCG2) localized in the apical/luminal membrane of the brain capillary endothelial cells are a major barrier of brain penetration of drugs.

Functional studies have assigned a role for human MRP2 (ABCC2) in the blood-brain barrier. MRP1 (ABCC1) is also implicated in protecting the brain tissue against xenobiotics (*e.g.* somatostatin analogs). MRP1 is localized in the basolateral membrane of the choroid epithelial cells and prevents the penetration of drugs and toxicants into the cephalo-spinal fluid. Similarity between the localization of MRP2, MXR (BCRP) and MDR1 in the brain microvessel endothelial cells and in the enteral epithelial cells suggests that these transporters function together to serve as physiological barriers against xenobiotics at the intestinal brush-border membrane and at the blood-brain barrier. MRP4 and MRP5 have been located in the brain capillary endothelial cells forming the blood-brain barrier. MRP1, MRP4, and MRP5 are clearly localized to the luminal side of brain capillary endothelial cells.

Despite advances in brain research, central nervous system (CNS) disorders remain very difficult to treat because the majority of drugs do not cross the BBB. The BBB blocks delivery of more than 98% of CNS acting drugs (*262,268*). Successful brain penetration is a prerequisite for the design of chemical lead substances for CNS acting drugs. To restrict CNS adverse effects, brain penetration properties are also important for the development of non-CNS acting drugs. Therefore, for both drug classes their BBB penetration is useful to be tested in advance. PgP and other ABC transporters can limit the penetration of drugs into the brain and thus modulate effectiveness and central nervous system toxicity of numerous drugs (*262,269,270*). The drug delivery challenge posed by the BBB is compelling, particularly as the population ages and the incidence of neurodegenerative diseases such as stroke, Alzheimer's disease, and Parkinson's disease increase in prevalence. Despite advances in brain research, central nervous system disorders remain very difficult to treat because the majority of drugs do not cross the BBB. The BBB limits the ability of many drugs to penetrate brain tissue by restricting paracellular and transcellular transport (*262*). To circumvent the limited access of drugs into the brain, different approaches have been investigated, including drug delivery systems such as liposomes, nanoparticles, peptide-vector strategy, MDR1 modulators, modulators of endothelial tight junctions, or osmotic pressure modification (*271*).

MRP alterations may also affect the distribution of their substrates, thus altering therapeutics or toxicology. For example, MRP4-deficient mice had enhanced accumulation of topotecan in brain tissue and cerebrospinal fluid (*166*). On the other hand, modulation of MRPs in blood-brain barrier may facilitate the management of diseases of the central nervous system by enhancing penetration of drugs into the brain. Such MRP-based barrier may be circumvented by targeted site-specific drug delivery systems, such

as immunoliposome and nanoparticulate systems (*272*). Moreover, development of novel approaches for bypassing the impact of these drug transporters and for the design of effective drugs that are not substrates and the development of selective and potent inhibitors for the MRP transporters becomes a high imperative for the pharmaceutical industry (*269*).

MRPs enhanced the ability of tumor cells to efflux chemotherapy drugs out of cells to reduce the cellular drug concentration leading to resistance to anticancer drugs. Increased expression of these drug transporters in tumor cells is associated with resistance to a number of important chemotherapeutic agents. With the accumulation of information on drug resistance profile and physiological function of MRP family, the relationship between drug selectivity and specific transporter level will be more and more significant and helpful in clinical cancer treatment and development of novel anticancer agents.

MRPs can be regulated at the level of transcription, translation and post-translation. Like PgP, MRPs are also subject to induction and inhibition by a number of compounds. Not surprisingly, the induction and inhibition of MRPs by various agents are of pharmacokinetic and pharmacodynamic importance. The identification of induces and inhibitors for each MRP may also allow the prediction of potential drug-drug interactions.

9. MRPs and drug toxicity

MRPs can efflux the GSH conjugated xenobiotics and endobiotics from the intracellular compartment into extracellular medium. This can protect cells from the toxic effects of xenobiotics and endobiotics. Therefore, screening the substrates and inhibitors of MRPs could point out the physiological function for each member of MRPs. Also, this could give information on toxicity and efficacy of individual drug. Modulation of MRPs activity seems to be significant to find new mechanism of drug-drug interaction and optimize drug bioavailability.

In addition to playing an important role in drug excretion through the bile, MRPs serve as protective shields by preventing uptake or facilitating clearance of toxic substances in the liver. Anti-toxic effects of MRP1-3 have been studied in more details. MRP2 (ABCC2) is involved in hepato-biliary excretion of GSH conjugates of inorganic arsenic and its chemical derivatives. In addition, some food-derived carcinogens and pre-carcinogens and their glucuronide conjugates are also transported by MRP2 (ABCC2), MRP1 (ABCC1) and MRP3 (ABCC3) may also contribute to the toxicological defense function by eliminating a number of toxic agents and their conjugates from epithelial tissues. It has been observed, that MRP3 (ABCC3) expression is strongly upregulated in the liver of the MRP2 (ABCC2) deficient patients and animals implying that basolateral MRP1 and MRP3-mediated efflux of toxicants may become of pivotal importance when administering MRP2-interacting drugs. ABC pumps play important function in the homeostasis of their own endogenous substrates. At pharmacological blockade of the transport, endogenous substrates may cause toxicity and adverse effects. MRP2 (ABCC2), which transports sulfated bile salts as well as bilirubin conjugates, and MDR3 (ABCB4), the phosphatidyl choline flippase, in particular carry important functions, therefore full or partial blockade of these proteins may evoke toxicity and adverse effects.

10. Pharmacogenetics of MRPs

In vitro site-directed mutagenesis studies indicate that mutants of MRPs may exhibit an altered substrate specificity, plasma membrane trafficking, ATP binding and transport activity (*12*,*273*-*275*). The replacement of Glu^{1089} with a neutral or positive charged amino acid reduced or completely eliminated the anthracycline resistance of MRP1 without influencing transport of LTC_4 and $E_2$17βG (*12*). Substitution of the aromatic residue (Trp^{653} in NBD1 and Tyr^{1302} in NBD2) with a polar cysteine residue, such as W653C or Y1302C, decreased the affinity for ATP, resulting in greatly increased K_d values for ATP binding or K_m values for ATP in ATP-dependent LTC_4 transport (*273*). In addition, the mutation N597A near transmembrane helix increased and decreased resistance to vincristine and VP-16, respectively, while S605A decreased resistance to vincristine, VP-16 and doxorubicin and S604A selectively increased $E_2$17βG transport (*274*).

A number of mutations in MRP1 have been found in different ethnic populations (Table 2), but these are not associated with any known genetic diseases. Nevertheless, some of these *MRP1* mutations may be associated with altered drug disposition. Substitution of Arg^{433} with Ser predicted to be close to TM8 of MRP1 caused by the low frequency G1299T polymorphism in exon 10 leads to a substrate selective change in organic anion transport activity and drug resistance using MRP1-expressing HeLa cells (*276*) or human leukemia CEM-7A cells (*277*). The 128C *MRP1* polymorphism in exon 2 resulting in Cys43Ser substitution disrupted plasma membrane trafficking and reduced resistance to doxorubicin, vincristine and arsenite in HeLa cells expressing this MRP1 mutant while the transport of conjugated organic anion remained comparable to wild type MRP1 (*278*,*279*). Further studies are needed to explore the pharmacological role of *MRP1* polymorphism in humans.

Spontaneous mutant strains of hyperbilirubinemic rat, the Groningen yellow/transport deficient Wistar rat and the Eisai hyperbilirubinemic Sprague-Dawley rat are deficient in biliary excretion of bilirubin glucuronides and glucuronide and glutathione conjugates of xenobiotics due to mutations of *Mrp2*

Table 2. Important single nucleotide polymorphisms (SNPs) of *MRP* genes

MRP genes	Chromosomal location	Amino acid variation	Nucleotide variation	Location	Reference
MRP1	16p13.11 - p13.12	Cys43Ser	G128C	Exon2	*287*
		Thr73Ile	C218T	Exon2	
		Arg433Ser	G1299T	Exon10	*276*
		Gly671Val	G2012T	Exon16	*307*
		Arg723Gln	G2168A	Exon17	*287*
		Arg1058Gln	G3173A	Exon23	*287*
MRP2	10q23 - 24		C-24T	Promoter	*118*, *287*
		Val417Ile	G1249A	Exon10	*118*, *286*, *287*
		Gly676Arg	G2026C	Exon16	*285*
		Try709Arg	T2125C	Exon17	*284*
		Arg768Trp	C2302T	Exon18	*118*, *286*, *287*
		Ser789Phe	C2366T	Exon18	
		I1173F	A3517T	Exon25	*288*
		R1150H	G3449A	Exon25	
		Ile1324Ile	C3972T	Exon28	*118*, *287*
		Ala1450Thr	G4348A	Exon31	*118*, *286*, *287*
MRP3	17q21.3	Lys13Asn	G39GC	Exon1	*290*
		His68Tyr	C202T	Exon2	
		Ser346Phe	C1037T	Exon9	
		Gln513Lys	C1537A	Exon12	
		Arg1297His	G3890A	Exon27	
		Gly1423Arg	G4267A	Exon29	
MRP4	13q32.1	Unknown	Unknown	Unknown	
MRP5	3q27	Unknown	Unknown	Unknown	
MRP6	16p13.1	L63L	G189G > C	Exon2	*299*
		W64R	190T > C	Exon2	
		T364R	1091C > G	Exon9	*308*, *309*
		Q378X	1132C > T	Exon9	
		R518X	1552 C > T	Exon12	*296*, *310*
		R518Q	1553G > A	Exon12	
		R1141X	3421C > T	Exon24	*295*, *296*
		R1138Q	3413G > A	Exon24	
		T1130M	3389C > T	Exon24	
		R1114C	3340C > T	Exon24	
		M1127T	3380C > T	Exon24	
		R1275X	3823C > T	Exon27	*295*
		P1346S	4036C > T	Exon28	*295*
		E1400K	4198G > A	Exon29	*296*
MRP7	6p12 - 21	Unknown	Unknown	Unknown	
MRP8	16q12.1	Unknown	Unknown	Unknown	
MRP9	16q12.1	Unknown	Unknown	Unknown	

gene (*280-282*). Such mutations in the *Mrp2* gene cause premature termination codons. Cloning of *mrp2* has made possible an understanding of its structure-function relationships, localization and regulation of expression, and characterization of the defect in patients with the Dubin-Johnson Syndrome (DJS). Mutations of MRP2 are responsible for DJS, which is characterized with impairment of hepatobiliary elimination of organic anions such as conjugated hyperbilirubinaemia, increased urinary coproporphyrin I fraction (> 80%), and deposition of melanin-like pigment in the liver (*105*,*282*,*283*). Patients with DJS may also have a decreased biliary clearance of bromosulfophthalein and some degree of jaundice (*105*). The absence of functional MRP2 is the molecular basis of transport defect of DJS (*283*). Many single nucleotide polymorphisms in DJS patients have been reported (Table 2) (*118*,*284-288*). These include C-24T (promoter), G1249A (exon 10), G2026C (exon 16), T2125C (exon 17), C2302T (exon 18), C2366 (exon 18), A3517T (exon 25), G3449 (exon 25), C3972T (exon 28) and G4348A (Exon 31) (Table 2). Many of these mutations are localized to NBD1 or NBD2. For instance, G4348A may affect MRP2 function because it is located in the *Walker C* motif within the carboxyl terminal NBD region of MRP2 (*118*). S789F and A1450T which are less frequently than V417I substitution may be more relevant to the *in vivo* function of MRP2 than V417I (*286*). Homozygous mutations lead to classic Dubin-Johnson syndrome, whereas heterozygous mutants have moderately elevated urinary coproporphyrin 1 fraction (~40%) with normal total and direct bilirubin (*105*). Unlike other mutations, R1150H mutants of the MRP2 protein mature and are properly localized, but transport activity is impaired (*288*). In addition, a significant allelic association between the 1249G > A SNP in *MRP2* gene and tenofovir-induced tenofovir-induced proximal

tubulopathy (*289*). Future studies are needed to identify any polymorphisms and their impact on MRP2 function.

Lang *et al*. (*290*) have reported the *MRP3* gene polymorphisms in 103 Caucasians. A total of 51 mutations were identified and 15 SNPs were located in the coding exons of MRP3, six of which are nonsynonymous mutations. The SNPs G39GC (allele frequency = 0.5%, in exon 1), C202T (1.6%, exon 2), C1037T (0.5%, exon 9), C1537A (0.5%, exon 12), G3890A (5.2%, exon 27) and G4267A (0.6%, exon 29) led to Lys13Asn, His68Tyr, Ser346Phe, Gln513Lys, Arg1297His and Gly1423Arg amino acid substitutions, respectively (Table 2). A splice site mutation (G1339-1T) was found at the intron 10-exon 11 boundary. There was a significant correlation of C-211T with *MRP3* mRNA expression, with individuals homozygous and heterozygous for the C-211T promoter polymorphism having significantly lower *MRP3* transcript levels compared to wild-type individuals.

Pseudoxanthoma elasticum (PXE) is an autosomally inherited disorder characterized by accumulation of mineralized and fragmented elastic fibers in the skin, Bruch's membrane in the retina, and vessel walls with abnormalities of collagen and matrix constituents in the soft connective tissues (*291-293*). The ophthalmic and dermatologic expression of PXE and its vascular complications are heterogeneous, with considerable variation in phenotype, progression, and mode of inheritance. Clinical manifestations mainly include coalesced papules and laxity in the flexural areas of skin, retinal angioid streaks and recurrent hemorrhage and vessel alterations similar to those in atherosclerosis (*294*). Lower expression of MRP6 was found in tissues affected by PXE, including skin, retina, and vessel walls. PXE is considered to be caused by mutations in *MRP6*. Small peptides transported by MRP6 in humans may be essential for extracellular matrix deposition or turnover of connective tissue at specific sites in the body.

Mutant alleles of *MRP6* occurred in homozygous, compound heterozygous and heterozygous forms. The great majority of mutations were located from exon 24 to 30, with exon 24 being the most affected (*295-298*). Among the others, exons 2, 9, and 12 were particularly involved (*295,299*). Almost all mutations were located in the intracellular site of MRP6.

A physiological function has only been established for MRP8, for which a single nucleotide polymorphism determines wet *vs* dry earwax type (*189*). However, the constituent of earwax that is susceptible to transport by MRP8 has not been identified. The functional characteristics and its genetic mutations of MRP9 are currently unknown.

Since MRPs are able to transport a wide range of drugs with various structures, the analysis of polymorphisms of these drug transporters may provide a potent tool for improving the risk assessment, prevention, early diagnosis and treatment of diseases. Naturally occurring mutations in MRP/ABCC-related drug transporters have been reported, some of which are non-synonymous single nucleotide polymorphisms (*275*). The consequences of the resulting amino acid changes can sometimes be predicted from *in vitro* site-directed mutagenesis studies or from knowledge of mutations of analogous (conserved) residues in ABCC proteins that cause DJS, PXE (ABCC6), cystic fibrosis (CFTR/ABCC7) or persistent hyperinsulinemic hypoglycemia of infancy (SUR1/ABCC8) (*275*). Polymorphisms of MRPs could be recognized as an important source of interindividual variability of pharmacokinetics and pharmacodynamics of many drugs. Also, this could help to establish a more powerful patient orientated drug therapy against severe adverse effects and for better therapeutic outcome. Eventually, this may provide a powerful tool for drug development, particularly for those with a narrow therapeutic window, such as anticancer drugs.

11. MRPs as potential therapeutic targets in multidrug resistance

PgP-mediated or classic multidrug resistance (MDR), which was identified in the 1970s, is a well-characterized experimental phenomenon. Classic MDR is characterized by: a) cross-resistance between a series of chemically unrelated drugs, b) decreased drug accumulation in cancer cells, c) increased expression of PgP, and d) reversal of the phenotype by a variety of different compounds (*300*). The drugs most often involved in PgP-mediated MDR are of fungal or plant origin, including the anthracyclines (*e.g*. primarily daunorubicin and doxorubicin) and vinca alkaloids. Apart from drugs within these groups, a number of other, nonrelated compounds are able to induce PgP-mediated MDR (*e.g*., epipodophyllotoxins, actinomycin D, colchicine, the taxanes, and the anthracenedione derivatives (*300*). All these drugs are hydrophobic, and most are weak bases.

MRP members play an important role in cancer chemotherapy. The differences in substrate selectivity, organ distribution, and membrane localization of these pumps play major function in related cancer drug transports. The knowledge about the mechanism of drug resistance may be useful in predicting the human response of chemotherapy. The overlapping substrates range of MRPs may have significant contributions for the clinical use of modulators aimed to block the resistant activity of pumps and increase the intracellular drug levels.

Most compounds that efficiently block PgP have only low affinity for MRP1, MRP2 and other MRPs. Despite that there are only a few effective and specific MRP inhibitors available, drug targeting of these transporters may play a role in cancer chemotherapy and in the pharmacokinetics of substrate drugs (*301*). The perfect reversing agent is efficient, lacks unrelated pharmacological effects, shows no pharmacokinetic interactions with other drugs, tackles specific

mechanisms of resistance with high potency and is readily administered to patients. Selective down-regulation of resistance genes in cancer cells by antisense or interfering RNA is an emerging approach in therapeutics. Because there is sufficient evidence to implicate several MRPs as negative prognostic markers during cancer chemotherapy, the pharmacological reversal of MRP1 function becomes a possible approach for overcoming tumor resistance. Disulfiram, a drug approved for use in treating alcoholism, reverses either MDR1- or MRP1-mediated efflux of fluorescent drug substrates *via* inhibiting ATP hydrolysis and the binding of [α-^{32}P]8-azidoATP to P-glycoprotein and MRP1 (*302*).

Design of novel anticancer agents that evade transporter-mediated efflux is a potential approach to avoid multidrug resistance. Epothilones are novel microtubule-targeting agents with a paclitaxel-like mechanism of action that are not recognized by PgP, providing proof of the concept that new classes of anticancer agents that do not interact with the multidrug transporters can be developed to improve response to therapy. As most anticancer agents subject to efflux are currently irreplaceable in chemotherapy regimens, an attractive solution would be to chemically modify their susceptibility to being transported while retaining antineoplastic activity. Although such modifications frequently decrease the bioavailability or efficacy of drugs, some novel agents have been developed using this approach (*303*). The intracellular concentration of drugs can also be elevated by increasing the rate of influx by improving the formulation. Encapsulation of doxorubicin in polyethylene glycol-coated liposomes might be safer and occasionally more effective than conventional doxorubicin (*304*). Overexpression of ABC transporters, particularly PgP, BCRP and MRPs, has consistently been implicated as a cause for MDR both *in vitro* and *in vivo*.

New and effective strategies are needed to engage, evade or exploit these transporters to improve cancer therapy.

12. Conclusions and future directions

MRPs which belong to the ABC transporter family are able to transport a remarkable array of diverse endo- and xenobiotics and their metabolites. MRP1, MRP2 and MRP3 are lipophilic anion transporters with similar substrate ranges and confer resistance to some natural compounds and methotrexate. MRP4, MRP5, and MRP8 are cyclic nucleotide transporters. Each member of MRP family has its own specified substrates. Notably, the 190 kDa MRP and PgP only have 15% the same amino acid and differ greatly in many aspects. Substrates for PgP are mainly neutral or mildly positive lipophilic compounds, while MRP is able to pump conjugated organic anions and neutral organic compounds.

Differences in substrate range, subcellular localization, expression profiles and kinetic parameters of transport dictate distinct physiological functions for MRPs (*4*). For example, MRP1 is distinguished from MRP2 and MRP3 by its higher affinity for LTC_4, a feature that is reflected in the specific role that MRP1 plays in mediating immune responses involving cellular export of this cystinyl leukotriene (*41*). By contrast with MRP1, MRP2 is primarily expressed at canalicular (apical) surfaces of hepatocytes where it functions in the extrusion of endogenous organic anions such as bilirubin glucuronide and certain anticancer agents and in the provision of the biliary fluid constituent glutathione. In addition to the transport of glutathione and glucuronate conjugates, MRP3 has the additional capability of mediating the transport of monoanionic bile acids. The latter feature, in combination with its induction at basolateral surfaces of hepatocytes and cholangiocytes under cholestatic conditions, support the notion that it functions as a compensatory backup mechanism to eliminate from these cells potentially toxic compounds that are ordinarily excreted into the bile. With regard to drug-resistance capabilities, MRP1, MRP2, and MRP3 are able to confer cellular resistance to natural product agents to varying extents, and all three pumps are potent methotrexate resistance factors (*9*). Recent investigations of MRP4 and MRP5 indicate that they have the facility for mediating the transport of cyclic nucleotides, a property that has implicated the two pumps in the regulation of intracellular levels of these second messengers as well as in the cellular extrusion of cAMP involved in intercellular signalling (*4*). In accord with their capacity to transport cyclic nucleotides, MRP4 and MRP5 have the facility for conferring resistance to certain antiviral and anticancer nucleotide analogs but do not seem to be capable of effluxing natural product agents (*9*). MRP6, whose hereditary deficiency results in PXE, a disease that affects elastic tissues in the skin, eyes, and cardiovascular system, has recently been determined to be competent in the transport of glutathione conjugates and the cyclic pentapeptide BQ123 (*182*). MRP7 was able to catalyze the MgATP-energized transport of the glucuronide $E_2$17βG. By comparison with $E_2$17βG, only modest transport was observed for LTC_4, and transport of a range of other compounds that are established substrates of other MRP family members was not detected to any extent (*184*). Further studies are needed to elucidate the clinical, pharmacological and toxicological relevance of all these MRPs.

Interindividual differences of drug response are an important cause of treatment failures and adverse drug reactions. The identification of polymorphisms explaining distinct phenotypes of drug metabolizing enzymes contributed in part to the understanding of individual variations of drug plasma levels. However, bioavailability also depends on a major extent from the expression and activity of drug transport across cellular membranes. In particular, the ABC family such as PgP/ABCB1, MRPs and BCRP/ABCG2 have been identified as major determinants of chemoresistance in

tumor cells. They are expressed in the apical membranes of many barrier tissues such as the intestine, liver, blood-brain barrier, kidney, placenta, testis and in lymphocytes, thus contributing to plasma, liquor, but also intracellular drug disposition (*305*). Since expression and function exhibit a broad variability, it was hypothesized that hereditary variances in the genes of ABC transporters could explain at least in part interindividual differences of pharmacokinetics and clinical outcome of a variety of drugs (*275*,*305*). The pharmacogenetic studies on MRPs including the single nucleotide polymorphism may provide powerful tools for drug development. Studies on the functions of MRPs may give more information on drug toxicity and drug-drug interaction. Continual updating of databases of sequence variants and haplotype analysis, together with *in vitro* biochemical validation assays and pharmacological studies in knockout animals, should make it possible to determine how genetic variation in the MRP-related transporters contributes to the range of responses to drugs and chemicals observed in different human populations. However, the mechanisms of MRPs activity and the substrates of some members of MRP family are unclear. In the future, we need to do more molecular, proteomic and genetic studies on MRPs to identify the regulation mechanism for individual MRPs.

The ability of transport proteins including MDR1, BCRP and MRPs to reduce oral bioavailability and alter tissue distribution has obvious implications for drug design (*306*). Indeed, the identification of transporters that influence the disposition and safety of drugs has become a new challenge for drug discovery programmes. It is essential to know, first, whether drugs can freely cross pharmacological barriers or whether their passage is restricted by ABC transporters; and, second, whether drugs can influence the passage of other compounds through the inhibition of ABC transporters. Consequently, the evaluation of transport susceptibility of drug candidates has become an important step in the development of novel therapeutics, and the pharmaceutical industry has adopted routine evaluation of PgP susceptibility in the drug discovery process. In the early stages of drug development, it is important to identify drugs as substrates, inducers, inhibitors, or modulators for MRPs, as this may help to avoid drug toxicity, drug resistance and drug-drug interactions and to optimize cancer chemotherapy. The identification always involves the application of proper models and probes, such as *in vitro* (*e.g*. purified MRP protein or MRP-overexpressing cells) and *in vivo* models.

References

1. Locher KP. Structure and mechanism of ABC transporters. Curr Opin Struct Biol 2004; 14:426-443.
2. Tian Q, Zhang J, Chan E, Duan W, Zhou SF. Multidrug resistance proteins (MRPs) and implication in drug development. Drug Dev Res 2005; 51:1-18.
3. Piddock LJ. Multidrug - resistance efflux pumps - not just for resistance. Nat Rev Microbiol 2006; 4:629-636.
4. Borst P, Elferink RO. Mammalian ABC transporters in health and disease. Annu Rev Biochem 2002; 71:537-592.
5. Dean M, Rzhetsky A, Allikmets R. The human ATP-binding cassette (ABC) transporter superfamily. Genome Res 2001; 11:1156-1166.
6. Müller M. 49 Human ATP-Binding Cassette Transporters. *http://www.nutrigene.4t.com/humanabc.htm*. (access date: December 5, 2008).
7. Jones PM, George AM. The ABC transporter structure and mechanism: perspectives on recent research. Cell Mol Life Sci 2004; 61:682-699.
8. Klein I, Sarkadi B, Váradi A. An inventory of the human ABC proteins. Biochim Biophys Acta 1999; 1461:237-262.
9. Borst P, Evers R, Kool M, Wijnholds J. A family of drug transporters: the multidrug resistance-associated proteins. J Natl Cancer Inst 2000; 92:1295-1302.
10. Walker JE, Saraste M, Runswick MJ, Gay NJ. Distantly related sequences in the alpha- and beta-subunits of ATP synthase, myosin, kinases and other ATP-requiring enzymes and a common nucleotide binding fold. Embo J 1982; 1:945-951.
11. Chang G, Roth CB. Structure of MsbA from *E. coli*: a homolog of the multidrug resistance ATP binding cassette (ABC) transporters. Science 2001; 293:1793-1800.
12. Zhang DW, Cole SP, Deeley RG. Identification of a nonconserved amino acid residue in multidrug resistance protein 1 important for determining substrate specificity: evidence for functional interaction between transmembrane helices 14 and 17. J Biol Chem 2001; 276:34966-34974.
13. Higgins CF. ABC transporters: from microorganisms to man. Annu Rev Cell Biol 1992; 8:67-113.
14. Altenberg GA. Structure of multidrug-resistance proteins of the ATP-binding cassette (ABC) superfamily. Curr Med Chem Anticancer Agents 2004; 4:53-62.
15. Kruh GD, Belinsky MG. The MRP family of drug efflux pumps. Oncogene 2003; 22:7537-7552.
16. Dean M, Rzhetsky A, Allikmets R. The human ATP-binding cassette (ABC) transporter superfamily. Genome Res 2001; 11:1156-1166.
17. Belinsky MG, Bain LJ, Balsara BB, Testa JR, Kruh GD. Characterization of MOAT-C and MOAT-D, new members of the MRP/cMOAT subfamily of transporter proteins. J Natl Cancer Inst 1998; 90:1735-1741.
18. Hopper E, Belinsky MG, Zeng H, Tosolini A, Testa JR, Kruh GD. Analysis of the structure and expression pattern of MRP7 (ABCC10), a new member of the MRP subfamily. Cancer Lett 2001; 162:181-191.
19. Bera TK, Lee S, Salvatore G, Lee B, Pastan I. MRP8, a new member of ABC transporter superfamily, identified by EST database mining and gene prediction program, is highly expressed in breast cancer. Mol Med 2001; 7:509-516.
20. Tammur J, Prades C, Arnould I, Rzhetsky A, Hutchinson A, Adachi M, Schuetz JD, Swoboda KJ, Ptácek LJ, Rosier M, Dean M, Allikmets R. Two new genes from the human ATP-binding cassette transporter superfamily, ABCC11 and ABCC12, tandemly duplicated on chromosome 16q12. Gene 2001; 273:89-96.
21. Yabuuchi H, Shimizu H, Takayanagi S, Ishikawa T. Multiple splicing variants of two new human ATP-binding cassette transporters, ABCC11 and ABCC12. Biochem

Biophys Res Commun 2001; 288:933-939.
22. Bakos E, Evers R, Szakács G, Tusnády GE, Welker E, Szabó K, de Haas M, van Deemter L, Borst P, Váradi A, Sarkadi B. Functional multidrug resistance protein (MRP1) lacking the *N*-terminal transmembrane domain. J Biol Chem 1998; 273: 32167-32175.
23. Cole SP, Bhardwaj G, Gerlach JH, Mackie JE, Grant CE, Almquist KC, Stewart AJ, Kurz EU, Duncan AM, Deeley RG. Overexpression of a transporter gene in a multidrug-resistant human lung cancer cell line. Science 1992; 258:1650-1654.
24. Cole SP, Deeley RG. Multidrug resistance mediated by the ATP-binding cassette transporter protein MRP. Bioessays 1998; 20:931-940.
25. Zaman GJ, Versantvoort CH, Smit JJ, Eijdems EW, de Haas M, Smith AJ, Broxterman HJ, Mulder NH, de Vries EG, Baas F, Borst P. Analysis of the expression of MRP, the gene for a new putative transmembrane drug transporter, in human multidrug resistant lung cancer cell lines. Cancer Res 1993; 53:1747-1750.
26. Burger H, Nooter K, Zaman GJ, Sonneveld P, van Wingerden KE, Oostrum RG, Stoter G. Expression of the multidrug resistance-associated protein (MRP) in acute and chronic leukemias. Leukemia 1994; 8:990-997.
27. Stride BD, Valdimarsson G, Gerlach JH, Wilson GM, Cole SP, Deeley RG. Structure and expression of the messenger RNA encoding the murine multidrug resistance protein, an ATP-binding cassette transporter. Mol Pharmacol 1996; 49:962-971.
28. Flens MJ, Zaman GJ, van der Valk P, Izquierdo MA, Schroeijers AB, Scheffer GL, van der Groep P, de Haas M, Meijer CJ, Scheper RJ. Tissue distribution of the multidrug resistance protein. Am J Pathol 1996; 148:1237-1247.
29. Wijnholds J, Scheffer GL, van der Valk M, van der Valk P, Beijnen JH, Scheper RJ, Borst P. Multidrug resistance protein 1 protects the oropharyngeal mucosal layer and the testicular tubules against drug-induced damage. J Exp Med 1998; 188:797-808.
30. Qian YM, Song WC, Cui H, Cole SP, Deeley RG. Glutathione stimulates sulfated estrogen transport by multidrug resistance protein 1. J Biol Chem 2001; 276:6404-6411.
31. Leslie EM, Deeley RG, Cole SP. Toxicological relevance of the multidrug resistance protein 1, MRP1 (ABCC1) and related transporters. Toxicology 2001; 167:3-23.
32. Lautier D, Canitrot Y, Deeley RG, Cole SP. Multidrug resistance mediated by the multidrug resistance protein (*MRP*) gene. Biochem Pharmacol 1996; 52:967-977.
33. Hammond CL, Marchan R, Krance SM, Ballatori N. Glutathione export during apoptosis requires functional multidrug resistance-associated proteins. J Biol Chem 2007; 282:14337-14347.
34. Leier I, Jedlitschky G, Buchholz U, Center M, Cole SP, Deeley RG, Keppler D. ATP-dependent glutathione disulphide transport mediated by the *MRP* gene-encoded conjugate export pump. Biochem J 1996; 314:433-437.
35. Loe DW, Almquist KC, Cole SP, Deeley RG. ATP-dependent 17 beta-estradiol 17-(beta-D-glucuronide) transport by multidrug resistance protein (MRP). Inhibition by cholestatic steroids. J Biol Chem 1996; 271:9683-9689.
36. Jedlitschky G, Leier I, Buchholz U, Barnouin K, Kurz G, Keppler D. Transport of glutathione, glucuronate, and sulfate conjugates by the *MRP* gene-encoded conjugate export pump. Cancer Res 1996; 56:988-994.
37. Rigato I, Pascolo L, Fernetti C, Ostrow JD, Tiribelli C. The human multidrug-resistance-associated protein MRP1 mediates ATP-dependent transport of unconjugated bilirubin. Biochem J 2004; 383:335-341.
38. Evers R, Cnubben NH, Wijnholds J, van Deemter L, van Bladeren PJ, Borst P. Transport of glutathione prostaglandin A conjugates by the multidrug resistance protein 1. FEBS Lett 1997; 419:112-116.
39. Akimaru K, Kuo MT, Furuta K, Suzuki M, Noyori R, Ishikawa T. Induction of MRP/GS-X pump and cellular resistance to anticancer prostaglandins. Cytotechnology 1996; 19:221-227.
40. Robbiani DF, Finch RA, Jäger D, Muller WA, Sartorelli AC, Randolph GJ. The leukotriene C(4) transporter MRP1 regulates CCL19 (MIP-3beta, ELC)-dependent mobilization of dendritic cells to lymph nodes. Cell 2000; 103:757-768.
41. Wijnholds J, Evers R, van Leusden MR, Mol CA, Zaman GJ, Mayer U, Beijnen JH, van der Valk M, Krimpenfort P, Borst P. Increased sensitivity to anticancer drugs and decreased inflammatory response in mice lacking the multidrug resistance-associated protein. Nat Med 1997; 3:1275-1279.
42. Cole SP, Sparks KE, Fraser K, Loe DW, Grant CE, Wilson GM, Deeley RG. Pharmacological characterization of multidrug resistant MRP-transfected human tumor cells. Cancer Res 1994; 54:5902-5910.
43. Zaman GJ, Flens MJ, van Leusden MR, de Haas M, Mülder HS, Lankelma J, Pinedo HM, Scheper RJ, Baas F, Broxterman HJ. The human multidrug resistance-associated protein MRP is a plasma membrane drug-efflux pump. Proc Natl Acad Sci U S A 1994; 91:8822-8826.
44. Versantvoort CH, Broxterman HJ, Bagrij T, Scheper RJ, Twentyman PR. Regulation by glutathione of drug transport in multidrug-resistant human lung tumour cell lines overexpressing multidrug resistance-associated protein. Br J Cancer 1995; 72:82-89.
45. Zaman GJ, Lankelma J, van Tellingen O, Beijnen J, Dekker H, Paulusma C, Oude Elferink RP, Baas F, Borst P. Role of glutathione in the export of compounds from cells by the multidrug-resistance-associated protein. Proc Natl Acad Sci U S A 1995; 92:7690-7694.
46. Rappa G, Lorico A, Flavell RA, Sartorelli AC. Evidence that the multidrug resistance protein (MRP) functions as a co-transporter of glutathione and natural product toxins. Cancer Res 1997; 57:5232-5237.
47. Cole SP, Downes HF, Mirski SE, Clements DJ. Alterations in glutathione and glutathione-related enzymes in a multidrug-resistant small cell lung cancer cell line. Mol Pharmacol 1990; 37:192-197.
48. Leier I, Jedlitschky G, Buchholz U, Cole SP, Deeley RG, Keppler D. The *MRP* gene encodes an ATP-dependent export pump for leukotriene C_4 and structurally related conjugates. J Biol Chem 1994; 269:27807-27810.
49. Müller M, Meijer C, Zaman GJ, Borst P, Scheper RJ, Mulder NH, de Vries EG, Jansen PL. Overexpression of the gene encoding the multidrug resistance-associated protein results in increased ATPdependent glutathione *S*-conjugate transport. Proc Natl Acad Sci U S A 1994; 91:13033-13037.
50. Loe DW, Deeley RG, Cole SP. Characterization of vincristine transport by the M(r) 190,000 multidrug resistance protein (MRP): evidence for cotransport with reduced glutathione. Cancer Res 1998; 58:5130-5136.
51. Salerno M, Garnier-Suillerot A. Kinetics of glutathione and daunorubicin efflux from multidrug resistance protein overexpressing small-cell lung cancer cells. Eur J

Pharmacol 2001; 421:1-9.
52. Leslie EM, Deeley RG, Cole SP. Bioflavonoid stimulation of glutathione transport by the 190-kDa multidrug resistance protein 1 (MRP1). Drug Metab Dispos 2003; 31:11-15.
53. Leslie EM, Ito K, Upadhyaya P, Hecht SS, Deeley RG, Cole SP. Transport of the beta-*O*-glucuronide conjugate of the tobacco-specific carcinogen 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL) by the multidrug resistance protein 1 (MRP1). Requirement for glutathione or a non-sulfur-containing analog. J Biol Chem 2001; 276:27846-27854.
54. Yang Z, Horn M, Wang J, Shen DD, Ho RJ. Development and characterization of a recombinant Madin-Darby canine kidney cell line that expresses rat multidrug resistance-associated protein 1 (rMRP1). AAPS PharmSci 2004; 6:E8.
55. Nunoya K, Grant CE, Zhang D, Cole SP, Deeley RG. Molecular cloning and pharmacological characterization of rat multidrug resistance protein 1 (mrp1). Drug Metab Dispos 2003; 31:1016-1026.
56. Leslie EM, Haimeur A, Waalkes MP. Arsenic transport by the human multidrug resistance protein 1 (MRP1/ABCC1). Evidence that a tri-glutathione conjugate is required. J Biol Chem 2004; 279:32700-32708.
57. Ito K, Olsen SL, Qiu W, Deeley RG, Cole SP. Mutation of a single conserved tryptophan in multidrug resistance protein 1 (MRP1/ABCC1) results in loss of drug resistance and selective loss of organic anion transport. J Biol Chem 2001; 276:15616-15624.
58. Zeng H, Chen ZS, Belinsky MG, Rea PA, Kruh GD. Transport of methotrexate (MTX) and folates by multidrug resistance protein (MRP) 3 and MRP1: effect of polyglutamylation on MTX transport. Cancer Res 2001; 61:7225-7232.
59. Hooijberg JH, Broxterman HJ, Kool M, Assaraf YG, Peters GJ, Noordhuis P, Scheper RJ, Borst P, Pinedo HM, Jansen G. Antifolate resistance mediated by the multidrug resistance proteins MRP1 and MRP2. Cancer Res 1999; 59:2532-2535.
60. Chu XY, Suzuki H, Ueda K, Kato Y, Akiyama S, Sugiyama Y. Active efflux of CPT-11 and its metabolites in human KB-derived cell lines. J Pharmacol Exp Ther 1999; 288:735-741.
61. Morrow CS, Smitherman PK, Diah SK, Schneider E, Townsend AJ. Coordinated action of glutathione *S*-transferases (GSTs) and multidrug resistance protein 1 (MRP1) in antineoplastic drug detoxification. Mechanism of GST A1-1- and MRP1-associated resistance to chlorambucil in MCF7 breast carcinoma cells. J Biol Chem 1998; 273:20114-20120.
62. Morrow CS, Peklak-Scott C, Bishwokarma B, Kute TE, Smitherman PK, Townsend AJ. Multidrug resistance protein 1 (MRP1, ABCC1) mediates resistance to mitoxantrone *via* glutathione-dependent drug efflux. Mol Pharmacol 2006; 69:1499-1505.
63. Paumi CM, Ledford BG, Smitherman PK, Townsend AJ, Morrow CS. Role of multidrug resistance protein 1 (MRP1) and glutathione *S*-transferase A1-1 in alkylating agent resistance. Kinetics of glutathione conjugate formation and efflux govern differential cellular sensitivity to chlorambucil *versus* melphalan toxicity. J Biol Chem 2001; 276:7952-7956.
64. Barnouin K, Leier I, Jedlitschky G, Pourtier-Manzanedo A, König J, Lehmann WD, Keppler D. Multidrug resistance protein-mediated transport of chlorambucil and melphalan conjugated to glutathione. Br J Cancer 1998; 77:201-209.
65. Lorico A, Rappa G, Finch RA, Yang D, Flavell RA, Sartorelli AC. Disruption of the murine *MRP* (*multidrug resistance protein*) gene leads to increased sensitivity to etoposide (VP-16) and increased levels of glutathione. Cancer Res 1997; 57:5238-5242.
66. Paulusma CC, van Geer MA, Evers R, Heijn M, Ottenhoff R, Borst P, Oude Elferink RP. Canalicular multispecific organic anion transporter/multidrug resistance protein 2 mediates low-affinity transport of reduced glutathione. Biochem J 1999; 338:393-401.
67. Meaden ER, Hoggard PG, Newton P, Tjia JF, Aldam D, Cornforth D, Lloyd J, Williams I, Back DJ, Khoo SH. P-glycoprotein and MRP1 expression and reduced ritonavir and saquinavir accumulation in HIV-infected individuals. J Antimicrob Chemother 2002; 50:583-588.
68. Williams GC, Liu A, Knipp G, Sinko PJ. Direct evidence that saquinavir is transported by multidrug resistance-associated protein (MRP1) and canalicular multispecific organic anion transporter (MRP2). Antimicrob Agents Chemother 2002; 46:3456-3462.
69. Dallas S, Ronaldson PT, Bendayan M, Bendayan R. Multidrug resistance protein 1-mediated transport of saquinavir by microglia. Neuroreport 2004; 15:1183-1186.
70. Grzywacz MJ, Yang JM, Hait WN. Effect of the multidrug resistance protein on the transport of the antiandrogen flutamide. Cancer Res 2003; 63:2492-2498.
71. Zaman GJ, Cnubben NH, van Bladeren PJ, Evers R, Borst P. Transport of the glutathione conjugate of ethacrynic acid by the human multidrug resistance protein MRP. FEBS Lett 1996; 391:126-130.
72. Hendrikse NH, Kuipers F, Meijer C, Havinga R, Bijleveld CM, van der Graaf WT, Vaalburg W, de Vries EG. *In vivo* imaging of hepatobiliary transport function mediated by multidrug resistance associated protein and P-glycoprotein. Cancer Chemother Pharmacol 2004; 54:131-138.
73. Chen WS, Luker KE, Dahlheimer JL, Pica CM, Luker GD, Piwnica-Worms D. Effects of MDR1 and MDR3 P-glycoproteins, MRP1, and BCRP/MXR/ABCP on the transport of (99m)Tc-tetrofosmin. Biochem Pharmacol 2000; 60:413-426.
74. Lorusso V, Pascolo L, Fernetti C, Visigalli M, Anelli P, Tiribelli C. *In vitro* and *in vivo* hepatic transport of the magnetic resonance imaging contrast agent B22956/1: role of MRP proteins. Biochem Biophys Res Commun 2002; 293:100-105.
75. Loe DW, Stewart RK, Massey TE, Deeley RG, Cole SP. ATP-dependent transport of aflatoxin B1 and its glutathione conjugates by the product of the multidrug resistance protein (*MRP*) gene. Mol Pharmacol 1997; 51:1034-1041.
76. Geisler M, Girin M, Brandt S, Vincenzetti V, Plaza S, Paris N, Kobae Y, Maeshima M, Billion K, Kolukisaoglu UH, Schulz B, Martinoia E. Arabidopsis immunophilin-like TWD1 functionally interacts with vacuolar ABC transporters. Mol Biol Cell 2004; 15:3393-3405.
77. Diah SK, Smitherman PK, Townsend AJ, Morrow CS. Detoxification of 1-chloro-2,4-dinitrobenzene in MCF7 breast cancer cells expressing glutathione *S*-transferase P1-1 and/or multidrug resistance protein 1. Toxicol Appl Pharmacol 1999; 157:85-93.
78. Morrow CS, Diah S, Smitherman PK, Schneider E, Townsend AJ. Multidrug resistance protein and glutathione *S*-transferase P1-1 act in synergy to confer protection from 4-nitroquinoline 1-oxide toxicity. Carcinogenesis 1998;

19:109-115.
79. Lorico A, Nesland J, Emilsen E, Fodstad O, Rappa G. Role of the multidrug resistance protein 1 gene in the carcinogenicity of aflatoxin B1: investigations using mrp1-null mice. Toxicology 2002; 171:201-205.
80. Johnson DR, Finch RA, Lin ZP, Zeiss CJ, Sartorelli AC. The pharmacological phenotype of combined multidrug-resistance mdr1a/1b- and mrp1-deficient mice. Cancer Res 2001; 61:1469-1476.
81. Gollapudi S, Kim CH, Tran BN, Sangha S, Gupta S. Probenecid reverses multidrug resistance in multidrug resistance-associated protein-overexpressing HL60/AR and H69/AR cells but not in P-glycoprotein-overexpressing HL60/Tax and P388/ADR cells. Cancer Chemother Pharmacol 1997; 40:150-158.
82. Draper MP, Martell RL, Levy SB. Indomethacin-mediated reversal of multidrug resistance and drug efflux in human and murine cell lines overexpressing MRP, but not P-glycoprotein. Br J Cancer 1997; 75:810-815.
83. Bakos E, Evers R, Sinkó E, Váradi A, Borst P, Sarkadi B. Interactions of the human multidrug resistance proteins MRP1 and MRP2 with organic anions. Mol Pharmacol 2000; 57:760-768.
84. Barrand MA, Rhodes T, Center MS, Twentyman PR. Chemosensitisation and drug accumulation effects of cyclosporin A, PSC-833 and verapamil in human MDR large cell lung cancer cells expressing a 190k membrane protein distinct from P-glycoprotein. Eur J Cancer 1993; 29A:408-415.
85. Hooijberg JH, Broxterman HJ, Heijn M, Fles DL, Lankelma J, Pinedo HM. Modulation by (iso)flavonoids of the ATPase activity of the multidrug resistance protein. FEBS Lett 1997; 413:344-348.
86. Hooijberg JH, Broxterman HJ, Scheffer GL, Vrasdonk C, Heijn M, de Jong MC, Scheper RJ, Lankelma J, Pinedo HM. Potent interaction of flavopiridol with MRP1. Br J Cancer 1999; 81:269-276.
87. Versantvoort CH, Broxterman HJ, Lankelma J, Feller N, Pinedo HM. Competitive inhibition by genistein and ATP dependence of daunorubicin transport in intact MRP overexpressing human small cell lung cancer cells. Biochem Pharmacol 1994; 48:1129-1136.
88. Nguyen H, Zhang S, Morris ME. Effect of flavonoids on MRP1-mediated transport in Panc-1 cells. J Pharm Sci 2003; 92:250-257.
89. Marbeuf-Gueye C, Salerno M, Quidu P, Garnier-Suillerot A. Inhibition of the P-glycoprotein- and multidrug resistance protein-mediated efflux of anthracyclines and calceinacetoxymethyl ester by PAK-104P. Eur J Pharmacol 2000; 391:207-216.
90. Aoki S, Chen ZS, Higasiyama K, Setiawan A, Akiyama S, Kobayashi M. Reversing effect of agosterol A, a spongean sterol acetate, on multidrug resistance in human carcinoma cells. Jpn J Cancer Res 2001; 92:886-895.
91. Bandi N, Kompella UB. Budesonide reduces multidrug resistance-associated protein 1 expression in an airway epithelial cell line (Calu-1). Eur J Pharmacol 2002; 437:9-17.
92. Payen L, Delugin L, Courtois A, Trinquart Y, Guillouzo A, Fardel O. Reversal of MRP-mediated multidrug resistance in human lung cancer cells by the antiprogestatin drug RU486. Biochem Biophys Res Commun 1999; 258:513-518.
93. Naito S, Koike K, Ono M, Machida T, Tasaka S, Kiue A, Koga H, Kumazawa J. Development of novel reversal agents, imidazothiazole derivatives, targeting MDR1- and MRP-mediated multidrug resistance. Oncol Res 1998; 10:123-132.
94. Weiss J, Theile D, Ketabi-Kiyanvash N, Lindenmaier H, Haefeli WE. Inhibition of MRP1/ABCC1, MRP2/ABCC2, and MRP3/ABCC3 by nucleoside, nucleotide, and non-nucleoside reverse transcriptase inhibitors. Drug Metab Dispos 2007; 35:340-344.
95. Gekeler V, Ise W, Sanders KH, Ulrich WR, Beck J. The leukotriene LTD4 receptor antagonist MK571 specifically modulates MRP associated multidrug resistance. Biochem Biophys Res Commun 1995; 208:345-352.
96. Nakano R, Oka M, Nakamura T, Fukuda M, Kawabata S, Terashi K, Tsukamoto K, Noguchi Y, Soda H, Kohno S. A leukotriene receptor antagonist, ONO-1078, modulates drug sensitivity and leukotriene C_4 efflux in lung cancer cells expressing multidrug resistance protein. Biochem Biophys Res Commun 1998; 251:307-312.
97. Payen L, Delugin L, Courtois A, Trinquart Y, Guillouzo A, Fardel O. The sulphonylurea glibenclamide inhibits multidrug resistance protein (MRP1) activity in human lung cancer cells. Br J Pharmacol 2001; 132:778-784.
98. Mao Q, Qiu W, Weigl KE, Lander PA, Tabas LB, Shepard RL, Dantzig AH, Deeley RG, Cole SP. GSH-dependent photolabeling of multidrug resistance protein MRP1 (ABCC1) by [^{125}I]LY475776. Evidence of a major binding site in the COOH-proximal membrane spanning domain. J Biol Chem 2002; 277:28690-28699.
99. Norman BH, Dantzig AH, Kroin JS, Law KL, Tabas LB, Shepard RL, Palkowitz AD, Hauser KL, Winter MA, Sluka JP, Starling JJ. Reversal of resistance in multidrug resistance protein (MRP1)-overexpressing cells by LY329146. Bioorg Med Chem Lett 1999; 9:3381-3386.
100. Qian YM, Grant CE, Westlake CJ, Zhang DW, Lander PA, Shepard RL, Dantzig AH, Cole SP, Deeley RG. Photolabeling of human and murine multidrug resistance protein 1 with the high affinity inhibitor [^{125}I]LY475776 and azidophenacyl-[^{35}S]glutathione. J Biol Chem 2002; 277:35225-35231.
101. Stewart AJ, Canitrot Y, Baracchini E, Dean NM, Deeley RG, Cole SP. Reduction of expression of the multidrug resistance protein (MRP) in human tumor cells by antisense phosphorothioate oligonucleotides. Biochem Pharmacol 1996; 51:461-469.
102. Peaston AE, Gardaneh M, Franco AV, Hocker JE, Murphy KM, Farnsworth ML, Catchpoole DR, Haber M, Norris MD, Lock RB, Marshall GM. *MRP1* gene expression level regulates the death and differentiation response of neuroblastoma cells. Br J Cancer 2001; 85:1564-1571.
103. Niewiarowski W, Gendaszewska E, Rebowski G, Wójcik M, Mikołajczyk B, Goss W, Soszyński M, Bartosz G. Multidrug resistance-associated protein--reduction of expression in human leukaemia cells by antisense phosphorothioate oligonucleotides. Acta Biochim Pol 2000; 47:1183-1188.
104. Evers R, Kool M, van Deemter L, Janssen H, Calafat J, Oomen LC, Paulusma CC, Oude Elferink RP, Baas F, Schinkel AH, Borst P. Drug export activity of the human canalicular multispecific organic anion transporter in polarized kidney MDCK cells expressing cMOAT (MRP2) cDNA. J Clin Invest 1998; 101:1310-1319.
105. Toh S, Wada M, Uchiumi T, Inokuchi A, Makino Y, Horie Y, Adachi Y, Sakisaka S, Kuwano M. Genomic structure of the canalicular multispecific organic anion-transporter gene (MRP2/cMOAT) and mutations in the ATP-binding-cassette region in Dubin-Johnson syndrome. Am J Human Genet 1999; 64:739-746.

106. Keppler D, Leier I, Jedlitschky G. Transport of glutathione conjugates and glucuronides by the multidrug resistance proteins MRP1 and MRP2. Biol Chem 1997; 378:787-791.
107. Masuda M, I'izuka Y, Yamazaki M, Nishigaki R, Kato Y, Ni'inuma K, Suzuki H, Sugiyama Y. Methotrexate is excreted into the bile by canalicular multispecific organic anion transporter in rats. Cancer Res 1997; 57:3506-3510.
108. Keppler D, Kartenbeck J. The canalicular conjugate export pump encoded by the cmrp/cmoat gene. Prog Liver Dis 1996; 14:55-67.
109. Cui Y, König J, Buchholz JK, Spring H, Leier I, Keppler D. Drug resistance and ATP-dependent conjugate transport mediated by the apical multidrug resistance protein, MRP2, permanently expressed in human and canine cells. Mol Pharmacol 1999; 55:929-937.
110. Kawabe T, Chen ZS, Wada M, Uchiumi T, Ono M, Akiyama S, Kuwano M. Enhanced transport of anticancer agents and leukotriene C_4 by the human canalicular multispecific organic anion transporter (cMOAT/MRP2). FEBS Lett 1999; 456:327-331.
111. Koike K, Kawabe T, Tanaka T, Toh S, Uchiumi T, Wada M, Akiyama S, Ono M, Kuwano M. A canalicular multispecific organic anion transporter (cMOAT) antisense cDNA enhances drug sensitivity in human hepatic cancer cells. Cancer Res 1997; 57:5475-5479.
112. Xiong H, Turner KC, Ward ES, Jansen PL, Brouwer KL. Altered hepatobiliary disposition of acetaminophen glucuronide in isolated perfused livers from multidrug resistance-associated protein 2-deficient TR(-) rats. J Pharmacol Exp Ther 2000; 295:512-518.
113. Evers R, de Haas M, Sparidans R, Beijnen J, Wielinga PR, Lankelma J, Borst P. Vinblastine and sulfinpyrazone export by the multidrug resistance protein MRP2 is associated with glutathione export. Br J Cancer 2000; 83:375-383.
114. Konno T, Ebihara T, Hisaeda K, Uchiumi T, Nakamura T, Shirakusa T, Kuwano M, Wada M. Identification of domains participating in the substrate specificity and subcellular localization of the multidrug resistance proteins MRP1 and MRP2. J Biol Chem 2003; 278:22908-22917.
115. Rebbeor JF, Connolly GC, Henson JH, Boyer JL, Ballatori N. ATP-dependent GSH and glutathione *S*-conjugate transport in skate liver: role of an Mrp functional homologue. Am J Physiol Gastrointest Liver Physiol 2000; 279:G417-425.
116. Paulusma CC, van Geer MA, Evers R, Heijn M, Ottenhoff R, Borst P, Oude Elferink RP. Canalicular multispecific organic anion transporter/multidrug resistance protein 2 mediates low-affinity transport of reduced glutathione. Biochem J 1999; 338:393-401.
117. Xiong H, Suzuki H, Sugiyama Y, Meier PJ, Pollack GM, Brouwer KL. Mechanisms of impaired biliary excretion of acetaminophen glucuronide after acute phenobarbital treatment or phenobarbital pretreatment. Drug Metab Dispos 2002; 30:962-969.
118. Suzuki H, Sugiyama Y. Single nucleotide polymorphisms in multidrug resistance associated protein 2 (MRP2/ABCC2): its impact on drug disposition. Adv Drug Deliv Rev 2002; 54:1311-1331.
119. Sasaki M, Suzuki H, Ito K, Abe T, Sugiyama Y. Transcellular transport of organic anions across a double-transfected Madin-Darby canine kidney II cell monolayer expressing both human organic anion-transporting polypeptide (OATP2/SLC21A6) and Multidrug resistance-associated protein 2 (MRP2/ABCC2). J Biol Chem 2002; 277:6497-6503.
120. Akita H, Suzuki H, Ito K, Kinoshita S, Sato N, Takikawa H, Sugiyama Y. Characterization of bile acid transport mediated by multidrug resistance associated protein 2 and bile salt export pump. Biochim Biophys Acta 2001; 1511:7-16.
121. Kobayashi N, Tani T, Hisaka A, Hara K, Yasumori T. Hepatobiliary transport of a nonpeptidic endothelin antagonist, (+)-(5*S*,6*R*,7*R*)-2-butyl-7-[2((2*S*)-2-carboxypropyl)-4-methoxyphenyl]-5-(3,4-methylenedioxyphenyl) cyclopentenol[1,2-*b*]pyridine-6-carboxylic acid: uptake by isolated rat hepatocytes and canalicular membrane vesicles. Pharm Res 2003; 20:89-95.
122. Nakagomi-Hagihara R, Nakai D, Kawai K, Yoshigae Y, Tokui T, Abe T, Ikeda T. OATP1B1, OATP1B3, and mrp2 are involved in hepatobiliary transport of olmesartan, a novel angiotensin II blocker. Drug Metab Dispos 2006; 34:862-869.
123. Takayanagi M, Sano N, Takikawa H. Biliary excretion of olmesartan, an anigotensin II receptor antagonist, in the rat. J Gastroenterol Hepatol 2005; 20:784-788.
124. Huisman MT, Smit JW, Crommentuyn KM, Zelcer N, Wiltshire HR, Beijnen JH, Schinkel AH. Multidrug resistance protein 2 (MRP2) transports HIV protease inhibitors, and transport can be enhanced by other drugs. Aids 2002; 16:2295-2301.
125. Agarwal S, Pal D, Mitra AK. Both P-gp and MRP2 mediate transport of Lopinavir, a protease inhibitor. Int J Pharm 2007; 339:139-147.
126. Hendrikse NH, Franssen EJ, van der Graaf WT, Vaalburg W, de Vries EG. Visualization of multidrug resistance *in vivo*. Eur J Nucl Med 1999; 26:283-293.
127. Srivastava SK, Watkins SC, Schuetz E, Singh SV. Role of glutathione conjugate efflux in cellular protection against benzo[a]pyrene-7,8-diol-9,10-epoxide-induced DNA damage. Mol Carcinog 2002; 33:156-162.
128. Terlouw SA, Graeff C, Smeets PH, Fricker G, Russel FG, Masereeuw R, Miller DS. Short- and long-term influences of heavy metals on anionic drug efflux from renal proximal tubule. J Pharmacol Exp Ther 2002; 301:578-585.
129. Dietrich CG, Ottenhoff R, de Waart DR, Oude Elferink RP. Role of MRP2 and GSH in intrahepatic cycling of toxins. Toxicology 2001; 167:73-81.
130. Bodo A, Bakos E, Szeri F, Varadi A, Sarkadi B. Differential modulation of the human liver conjugate transporters MRP2 and MRP3 by bile acids and organic anions. J Biol Chem 2003; 278:23529-23537.
131. Zelcer N, Huisman MT, Reid G, Wielinga P, Breedveld P, Kuil A, Knipscheer P, Schellens JH, Schinkel AH, Borst P. Evidence for two interacting ligand binding sites in human multidrug resistance protein 2 (ATP binding cassette C2). J Biol Chem 2003; 278:23538-23544.
132. Koike K, Kawabe T, Tanaka T, Toh S, Uchiumi T, Wada M, Akiyama S, Ono M, Kuwano M. A canalicular multispecific organic anion transporter (cMOAT) antisense cDNA enhances drug sensitivity in human hepatic cancer cells. Cancer Res 1997; 57:5475-5479.
133. Kiuchi Y, Suzuki H, Hirohashi T, Tyson CA, Sugiyama Y. cDNA cloning and inducible expression of human multidrug resistance associated protein 3 (MRP3). FEBS Lett 1998; 433:149-152.
134. Kool M, de Haas M, Scheffer GL, Scheper RJ, van Eijk MJ, Juijn JA, Baas F, Borst P. Analysis of expression of cMOAT (MRP2), MRP3, MRP4, and MRP5, homologues

of the multidrug resistance-associated protein gene (MRP1), in human cancer cell lines. Cancer Res 1997; 57:3537-3547.
135. Zimmermann C, Gutmann H, Hruz P, Gutzwiller JP, Beglinger C, Drewe J. Mapping of MDR1 and MRP1-5 mRNA expression along the human intestinal tract. Drug Metab Dispos 2005; 33:219-224.
136. Nies AT, Jedlitschky G, König J, Herold-Mende C, Steiner HH, Schmitt HP, Keppler D. Expression and immunolocalization of the multidrug resistance proteins, MRP1-MRP6 (ABCC1-ABCC6), in human brain. Neuroscience 2004; 129:349-360.
137. Kool M, van der Linden M, de Haas M, Scheffer GL, de Vree JM, Smith AJ, Jansen G, Peters GJ, Ponne N, Scheper RJ, Elferink RP, Baas F, Borst P. MRP3, an organic anion transporter able to transport anti-cancer drugs. Proc Natl Acad Sci U S A 1999; 96:6914-6919.
138. Zeng H, Bain LJ, Belinsky MG, Kruh GD. Expression of multidrug resistance protein-3 (multispecific organic anion transporter-D) in human embryonic kidney 293 cells confers resistance to anticancer agents. Cancer Res 1999; 59:5964-5967.
139. Zeng H, Liu G, Rea PA, Kruh GD. Transport of amphipathic anions by human multidrug resistance protein 3. Cancer Res 2000; 60:4779-4784.
140. Chu XY, Huskey SE, Braun MP, Sarkadi B, Evans DC, Evers R. Transport of ethinylestradiol glucuronide and ethinylestradiol sulfate by the multidrug resistance proteins MRP1, MRP2, and MRP3. J Pharmacol Exp Ther 2004; 309:156-164.
141. Zelcer N, Saeki T, Reid G, Beijnen JH, Borst P. Characterization of drug transport by the human multidrug resistance protein 3 (ABCC3). J Biol Chem 2001; 276:46400-46407.
142. Hirohashi T, Suzuki H, Sugiyama Y. Characterization of the transport properties of cloned rat multidrug resistance-associated protein 3 (MRP3). J Biol Chem 1999; 274:15181-15185.
143. Kool M, de Haas M, Scheffer GL, Scheper RJ, van Eijk MJ, Juijn JA, Baas F, Borst P. Analysis of expression of cMOAT (MRP2), MRP3, MRP4, and MRP5, homologues of the multidrug resistance-associated protein gene (MRP1), in human cancer cell lines. Cancer Res 1997; 57:3537-3547.
144. Young LC, Campling BG, Voskoglou-Nomikos T, Cole SP, Deeley RG, Gerlach JH. Expression of multidrug resistance protein-related genes in lung cancer: correlation with drug response. Clin Cancer Res 1999; 5:673-680.
145. Ishikawa T. The ATP-dependent glutathione *S*-conjugate export pump. Trends Biochem Sci 1993; 17:463-468.
146. Hirohashi T, Suzuki H, Takikawa H, Sugiyama Y. ATP-dependent transport of bile salts by rat multidrug resistance-associated protein 3 (Mrp3). J Biol Chem 2000; 275:2905-2910.
147. Kullak-Ublick GA, Stieger B, Meier PJ. Enterohepatic bile salt transporters in normal physiology and liver disease. Gastroenterology 2004; 126:322-342.
148. Ros JE, Libbrecht L, Geuken M, Jansen PL, Roskams TA. High expression of MDR1, MRP1, and MRP3 in the hepatic progenitor cell compartment and hepatocytes in severe human liver disease. J Pathol 2003; 200:553-560.
149. Zollner G, Fickert P, Silbert D, Fuchsbichler A, Marschall HU, Zatloukal K, Denk H, Trauner M. Adaptive changes in hepatobiliary transporter expression in primary biliary cirrhosis. J Hepatol 2003; 38:717-727.
150. Lai L, Tan TM. Role of glutathione in the multidrug resistance protein 4 (MRP4/ABCC4)-mediated efflux of cAMP and resistance to purine analogues. Biochem J 2002; 361:497-503.
151. Chen ZS, Lee K, Kruh GD. Transport of cyclic nucleotides and estradiol 17-beta-D-glucuronide by multidrug resistance protein 4. Resistance to 6-mercaptopurine and 6-thioguanine. J Biol Chem 2001; 276:33747-33754.
152. Chen ZS, Lee K, Walther S, Raftogianis RB, Kuwano M, Zeng H, Kruh GD. Analysis of methotrexate and folate transport by multidrug resistance protein 4 (ABCC4): MRP4 is a component of the methotrexate efflux system. Cancer Res 2002; 62:3144-3150.
153. van Aubel RA, Smeets PH, Peters JG, Bindels RJ, Russel FG. The *MRP4/ABCC4* gene encodes a novel apical organic anion transporter in human kidney proximal tubules: putative efflux pump for urinary cAMP and cGMP. J Am Soc Nephrol 2002; 13:595-603.
154. Reid G, Wielinga P, Zelcer N, van der Heijden I, Kuil A, de Haas M, Wijnholds J, Borst P. The human multidrug resistance protein MRP4 functions as a prostaglandin efflux transporter and is inhibited by nonsteroidal antiinflammatory drugs. Proc Natl Acad Sci U S A 2003; 100:9244-9249.
155. Rius M, Thon WF, Keppler D, Nies AT. Prostanoid transport by multidrug resistance protein 4 (MRP4/ABCC4) localized in tissues of the human urogenital tract. J Urol 2005; 174:2409-2414.
156. Zelcer N, Reid G, Wielinga P, Kuil A, van der Heijden I, Schuetz JD, Borst P. Steroid and bile acid conjugates are substrates of human multidrug-resistance protein (MRP) 4 (ATP-binding cassette C4). Biochem J 2003; 371:361-367.
157. Rius M, Nies AT, Hummel-Eisenbeiss J, Jedlitschky G, Keppler D. Cotransport of reduced glutathione with bile salts by MRP4 (ABCC4) localized to the basolateral hepatocyte membrane. Hepatology 2003; 38:374-384.
158. Zamek-Gliszczynski MJ, Nezasa K, Tian X, Bridges AS, Lee K, Belinsky MG, Kruh GD, Brouwer KL. Evaluation of the role of multidrug resistance-associated protein (Mrp) 3 and Mrp4 in hepatic basolateral excretion of sulfate and glucuronide metabolites of acetaminophen, 4-methylumbelliferone, and harmol in Abcc3$^{-/-}$ and Abcc4$^{-/-}$ mice. J Pharmacol Exp Ther 2006; 319:1485-1491.
159. Adachi M, Sampath J, Lan LB, Sun D, Hargrove P, Flatley R, Tatum A, Edwards MZ, Wezeman M, Matherly L, Drake R, Schuetz J. Expression of MRP4 confers resistance to ganciclovir and compromises bystander cell killing. J Biol Chem 2002; 277:38998-39004.
160. Sampath J, Adachi M, Hatse S, Naesens L, Balzarini J, Flatley RM, Matherly LH, Schuetz JD. Role of MRP4 and MRP5 in biology and chemotherapy. AAPS PharmSci 2002; 4:E14.
161. Lee K, Klein-Szanto AJ, Kruh GD. Analysis of the MRP4 drug resistance profile in transfected NIH3T3 cells. J Natl Cancer Inst 2000; 92:1934-1940.
162. Dallas S, Schlichter L, Bendayan R. Multidrug resistance protein (MRP) 4- and MRP 5-mediated efflux of 9-(2-pho sphonylmethoxyethyl)adenine by microglia. J Pharmacol Exp Ther 2004; 309:1221-1229.
163. Imaoka T, Kusuhara H, Adachi M, Schuetz JD, Takeuchi K, Sugiyama Y. Functional involvement of multidrug resistance-associated protein 4 (MRP4/ABCC4) in the renal elimination of the antiviral drugs adefovir and tenofovir. Mol Pharmacol 2007; 71:619-627.
164. Van Aubel RA, Smeets PH, van den Heuvel JJ, Russel FG. Human organic anion transporter MRP4 (ABCC4) is

an efflux pump for the purine end metabolite urate with multiple allosteric substrate binding sites. Am J Physiol Renal Physiol 2005; 288:F327-333.
165. Wielinga PR, Reid G, Challa EE, van der Heijden I, van Deemter L, de Haas M, Mol C, Kuil AJ, Groeneveld E, Schuetz JD, Brouwer C, De Abreu RA, Wijnholds J, Beijnen JH, Borst P. Thiopurine metabolism and identification of the thiopurine metabolites transported by MRP4 and MRP5 overexpressed in human embryonic kidney cells. Mol Pharmacol 2002; 62:1321-1331.
166. Leggas M, Adachi M, Scheffer GL, Sun D, Wielinga P, Du G, Mercer KE, Zhuang Y, Panetta JC, Johnston B, Scheper RJ, Stewart CF, Schuetz JD. Mrp4 confers resistance to topotecan and protects the brain from chemotherapy. Mol Cell Biol 2004; 24:7612-7621.
167. Wall ME, Wani MC, Cook CE, Palmer KH, McPhail AT, Sim GA. Plant antitumor agents: 1, the isolation and structure of camptothecin, anovel alkaloidal leukemia and tumor inhibitor from *Camptotheca acuminata*. J Am Chem Soc 1966; 88:3888-3890.
168. Bai J, Lai L, Yeo HC, Goh BC, Tan TM. Multidrug resistance protein 4 (MRP4/ABCC4) mediates efflux of bimane-glutathione. Int J Biochem Cell Biol 2004; 36:247-257.
169. Wielinga PR, van der Heijden I, Reid G, Beijnen JH, Wijnholds J, Borst P. Characterization of the MRP4- and MRP5-mediated transport of cyclic nucleotides from intact cells. J Biol Chem 2003; 278:17664-17671.
170. Assaraf YG, Sierra EE, Babani S, Goldman ID. Inhibitory effects of prostaglandin A1 on membrane transport of folates mediated by both the reduced folate carrier and ATP-driven exporters. Biochem Pharmacol 1999; 58:1321-1327.
171. Reid G, Wielinga P, Zelcer N, De Haas M, Van Deemter L, Wijnholds J, Balzarini J, Borst P. Characterization of the transport of nucleoside analog drugs by the human multidrug resistance proteins MRP4 and MRP5. Mol Pharmacol 2003; 63:1094-1103.
172. Schuetz EG, Strom S, Yasuda K, *et al*. Disrupted bile acid homeostasis reveals an unexpected interaction among nuclear hormone receptors, transporters, and cytochrome P450. J Biol Chem 2001; 276:39411-39418.
173. Assem M, Schuetz EG, Leggas M, Sun D, Yasuda K, Reid G, Zelcer N, Adachi M, Strom S, Evans RM, Moore DD, Borst P, Schuetz JD. Interactions between hepatic Mrp4 and Sult2a as revealed by the constitutive androstane receptor and *Mrp4* knockout mice. J Biol Chem 2004; 279:22250-22257.
174. Jorajuria S, Dereuddre-Bosquet N, Becher F, Martin S, Porcheray F, Garrigues A, Mabondzo A, Benech H, Grassi J, Orlowski S, Dormont D, Clayette P. ATP binding cassette multidrug transporters limit the anti-HIV activity of zidovudine and indinavir in infected human macrophages. Antivir Ther 2004; 9:519-528.
175. Wijnholds J, Mol CA, van Deemter L, de Haas M, Scheffer GL, Baas F, Beijnen JH, Scheper RJ, Hatse S, De Clercq E, Balzarini J, Borst P. Multidrug-resistance protein 5 is a multispecific organic anion transporter able to transport nucleotide analogs. Proc Natl Acad Sci U S A 2000; 97:7476-7481.
176. McAleer MA, Breen MA, White NL, Matthews N. pABC11 (also known as MOAT-C and MRP5), a member of the ABC family of proteins, has anion transporter activity but does not confer multidrug resistance when overexpressed in human embryonic kidney 293 cells. J Biol Chem 1999; 274:23541-23548.
177. Kool M, van der Linden M, de Haas M, Baas F, Borst P. Expression of human MRP6, a homologue of the multidrug resistance protein gene *MRP1*, in tissues and cancer cells. Cancer Res 1999; 59:175-182.
178. Scheffer GL, Hu X, Pijnenborg AC, Wijnholds J, Bergen AA, Scheper RJ. MRP6 (ABCC6) detection in normal human tissues and tumors. Lab Invest 2002; 82:515-518.
179. Boraldi F, Quaglino D, Croce MA, Garcia Fernandez MI, Tiozzo R, Gheduzzi D, Bacchelli B, Pasquali Ronchetti I. Multidrug resistance protein-6 (MRP6) in human dermal fibroblasts. Comparison between cells from normal subjects and from Pseudoxanthoma elasticum patients. Matrix Biol 2003; 22:491-500.
180. Sinkó E, Iliás A, Ujhelly O, Homolya L, Scheffer GL, Bergen AA, Sarkadi B, Váradi A. Subcellular localization and *N*-glycosylation of human ABCC6, expressed in MDCKII cells. Biochem Biophys Res Commun 2003; 308:263-269.
181. Belinsky MG, Chen ZS, Shchaveleva I, Zeng H, Kruh GD. Characterization of the drug resistance and transport properties of multidrug resistance protein 6 (MRP6, ABCC6). Cancer Res 2002; 62:6172-6177.
182. Madon J, Hagenbuch B, Landmann L, Meier PJ, Stieger B. Transport function and hepatocellular localization of mrp6 in rat liver. Mol Pharmacol 2000; 57:634-641.
183. Iliás A, Urbán Z, Seidl TL, Le Saux O, Sinkó E, Boyd CD, Sarkadi B, Váradi A. Loss of ATP-dependent transport activity in pseudoxanthoma elasticum-associated mutants of human ABCC6 (MRP6). J Biol Chem 2002; 277:16860-16867.
184. Chen ZS, Hopper-Borge E, Belinsky MG, Shchaveleva I, Kotova E, Kruh GD. Characterization of the transport properties of human multidrug resistance protein 7 (MRP7, ABCC10). Mol Pharmacol 2003; 63:351-358.
185. Hopper-Borge E, Chen ZS, Shchaveleva I, Belinsky MG, Kruh GD. Analysis of the drug resistance profile of multidrug resistance protein 7 (ABCC10): resistance to docetaxel. Cancer Res 2004; 64:4927-4930.
186. Oguri T, Bessho Y, Achiwa H, Ozasa H, Maeno K, Maeda H, Sato S, Ueda R. MRP8/ABCC11 directly confers resistance to 5-fluorouracil. Mol Cancer Ther 2007; 6:122-127.
187. Guo Y, Kotova E, Chen ZS, Lee K, Hopper-Borge E, Belinsky MG, Kruh GD. MRP8, ATP-binding cassette C11 (ABCC11), is a cyclic nucleotide efflux pump and a resistance factor for fluoropyrimidines 2',3'-dideoxycytidine and 9'-(2'-phosphonylmethoxyethyl) adenine. J Biol Chem 2003; 278:29509-29514.
188. Chen LM, Wu XP, Ruan JW, Liang YJ, Ding Y, Shi Z, Wang XW, Gu LQ, Fu LW. Screening novel, potent multidrug-resistant modulators from imidazole derivatives. Oncol Res 2004; 14:355-362.
189. Kruh GD, Guo Y, Hopper-Borge E, Belinsky MG, Chen ZS. ABCC10, ABCC11, and ABCC12. Pflugers Arch 2007; 453:675-684.
190. Bortfeld M, Rius M, König J, Herold-Mende C, Nies AT, Keppler D. Human multidrug resistance protein 8 (MRP8/ABCC11), an apical efflux pump for steroid sulfates, is an axonal protein of the CNS and peripheral nervous system. Neuroscience 2006; 137:1247-1257.
191. Chen ZS, Guo Y, Belinsky MG, Kotova E, Kruh GD. Transport of bile acids, sulfated steroids, estradiol 17-beta-D-glucuronide, and leukotriene C_4 by human multidrug resistance protein 8 (ABCC11). Mol Pharmacol 2005; 67:545-557.
192. Bera TK, Iavarone C, Kumar V, Lee S, Lee B, Pastan

I. MRP9, an unusual truncated member of the ABC transporter superfamily, is highly expressed in breast cancer. Proc Natl Acad Sci U S A 2002; 99:6997-7002.
193. Urquhart BL, Tirona RG, Kim RB. Nuclear receptors and the regulation of drug-metabolizing enzymes and drug transporters: implications for interindividual variability in response to drugs. J Clin Pharmacol 2007; 47:566-578.
194. Staudinger JL, Madan A, Carol KM, Parkinson A. Regulation of drug transporter gene expression by nuclear receptors. Drug Metab Dispos 2003; 31:523-527.
195. Klaassen CD, Slitt AL. Regulation of hepatic transporters by xenobiotic receptors. Curr Drug Metab 2005; 6:309-328.
196. Bertilsson G, Heidrich J, Svensson K, Asman M, Jendeberg L, Sydow-Bäckman M, Ohlsson R, Postlind H, Blomquist P, Berkenstam A. Identification of a human nuclear receptor defines a new signaling pathway for CYP3A induction. Proc Natl Acad Sci U S A 1998; 95:12208-12213.
197. Kliewer SA, Willson TM. Regulation of xenobiotic and bile acid metabolism by the nuclear pregnane X receptor. J Lipid Res 2000; 43:359-364.
198. Staudinger JL, Goodwin B, Jones SA, Hawkins-Brown D, MacKenzie KI, LaTour A, Liu Y, Klaassen CD, Brown KK, Reinhard J, Willson TM, Koller BH, Kliewer SA. The nuclear receptor PXR is a lithocholic acid sensor that protects against liver toxicity. Proc Natl Acad Sci U S A 2001; 98:3369-3374.
199. Geick A, Eichelbaum M, Burk O. Nuclear receptor response elements mediate induction of intestinal MDR1 by rifampin. J Biol Chem 2001; 276:14581-14587.
200. Kauffmann HM, Pfannschmidt S, Zöller H, Benz A, Vorderstemann B, Webster JI, Schrenk D. Influence of redox-active compounds and PXR-activators on human *MRP1* and *MRP2* gene expression. Toxicology 2002; 171:137-146.
201. Teng S, Jekerle V, Piquette-Miller M. Induction of ABCC3 (MRP3) by pregnane X receptor activators. Drug Metab Dispos 2003; 31:1296-1299.
202. Maher JM, Cheng X, Slitt AL, Dieter MZ, Klaassen CD. Induction of the multidrug resistance-associated protein family of transporters by chemical activators of receptor-mediated pathways in mouse liver. Drug Metab Dispos 2005; 33:956-962.
203. Moffit JS, Aleksunes LM, Maher JM, Scheffer GL, Klaassen CD, Manautou JE. Induction of hepatic transporters multidrug resistance-associated proteins (Mrp) 3 and 4 by clofibrate is regulated by peroxisome proliferator-activated receptor alpha. J Pharmacol Exp Ther 2006; 317:537-545.
204. Nishimura M, Koeda A, Suzuki E, Kawano Y, Nakayama M, Satoh T, Narimatsu S, Naito S. Regulation of mRNA expression of MDR1, MRP1, MRP2 and MRP3 by prototypical microsomal enzyme inducers in primary cultures of human and rat hepatocytes. Drug Metab Pharmacokinet 2006; 21:297-307.
205. Aleksunes LM, Scheffer GL, Jakowski AB, Pruimboom-Brees IM, Manautou JE. Coordinated expression of multidrug resistance-associated proteins (Mrps) in mouse liver during toxicant-induced injury. Toxicol Sci 2006; 89:370-379.
206. Magnarin M, Morelli M, Rosati A, Bartoli F, Candussio L, Giraldi T, Decorti G. Induction of proteins involved in multidrug resistance (P-glycoprotein, MRP1, MRP2, LRP) and of CYP 3A4 by rifampicin in LLC-PK1 cells. Eur J Pharmacol 2004; 483:19-28.
207. Pfrunder A, Gutmann H, Beglinger C, Drewe J. Gene expression of CYP3A4, ABC-transporters (MDR1 and MRP1-MRP5) and hPXR in three different human colon carcinoma cell lines. J Pharm Pharmacol 2003; 55:59-66.
208. Nieth C, Lage H. Induction of the ABC-transporters Mdr1/P-gp (Abcb1), mrpl (Abcc1), and bcrp (Abcg2) during establishment of multidrug resistance following exposure to mitoxantrone. J Chemother 2005; 17:215-223.
209. Tatebe S, Sinicrope FA, Kuo MT. Induction of multidrug resistance proteins MRP1 and MRP3 and gamma-glutamylcysteine synthetase gene expression by nonsteroidal anti-inflammatory drugs in human colon cancer cells. Biochem Biophys Res Commun 2002; 290:1427-1433.
210. Kauffmann HM, Schrenk D. Sequence analysis and functional characterization of the 5'-flanking region of the rat multidrug resistance protein 2 (*mrp2*) gene. Biochem Biophys Res Commun 1998; 245:325-331.
211. Johnson DR, Klaassen CD. Regulation of rat multidrug resistance protein 2 by classes of prototypical microsomal enzyme inducers that activate distinct transcription pathways. Toxicol Sci 2002; 67:182-189.
212. Courtois A, Payen L, Le Ferrec E, Scheffer GL, Trinquart Y, Guillouzo A, Fardel O. Differential regulation of multidrug resistance-associated protein 2 (MRP2) and cytochrornes P4502B1/2 and 3A1/2 in phenobarbital-treated hepatocytes. Biochem Pharmacol 2002; 63:333-341.
213. Cherrington NJ, Hartley DP, Li N, Johnson DR, Klaassen CD. Organ distribution of multidrug resistance proteins 1, 2, and 3 (Mrp1, 2 and 3) mRNA and hepatic induction of Mrp3 by constitutive androstane receptor activators in rats. J Pharmacol Exp Ther 2002; 300:97-104.
214. Kast HR, Goodwin B, Tarr PT, Jones SA, Anisfeld AM, Stoltz CM, Tontonoz P, Kliewer S, Willson TM, Edwards PA. Regulation of multidrug resistance-associated protein 2 (ABCC2) by the nuclear receptors pregnane X receptor, farnesoid X-activated receptor, and constitutive androstane receptor. J Biol Chem 2002; 277:2908-2915.
215. Kauffmann HM, Keppler D, Kartenbeck J, Schrenk D. Induction of *cMrp/cMOAT* gene expression by cisplatin, 2-acetylaminofluorene, or cycloheximide in rat hepatocytes. Hepatology 1997; 26:980-985.
216. Slitt AL, Cherrington NJ, Fisher CD, Negishi M, Klaassen CD. Induction of genes for metabolism and transport by trans-stilbene oxide in livers of Sprague-Dawley and Wistar-Kyoto rats. Drug Metab Dispos 2006; 34:1190-1197.
217. Kauffmann HM, Keppler D, Gant TW, Schrenk D. Induction of hepatic *MRP2* (*cMRP/cMOAT*) gene expression in nonhuman primates treated with rifampicin or tamoxifen. Arch Toxicol 1998; 72:763-768.
218. Rühl R, Sczech R, Landes N, Pfluger P, Kluth D, Schweigert FJ. Carotenoids and their metabolites are naturally occurring activators of gene expression *via* the pregnane X receptor. Eur J Nutr 2004; 43:336-343.
219. Jones SA, Moore LB, Shenk JL, Wisely GB, Hamilton GA, McKee DD, Tomkinson NC, LeCluyse EL, Lambert MH, Willson TM, Kliewer SA, Moore JT. The pregnane x receptor: a promiscuous xenobiotic receptor that has diverged during evolution. Mol Endocrinol 2000; 14:27-39.
220. Schrenk D, Baus PR, Ermel N, Klein C, Vorderstemann B, Kauffmann HM. Up-regulation of transporters of the MRP family by drugs and toxins. Toxicol Lett 2001; 120:51-57.
221. Shoda J, Kano M, Oda K, Kamiya J, Nimura Y, Suzuki

H, Sugiyama Y, Miyazaki H, Todoroki T, Stengelin S, Kramer W, Matsuzaki Y, Tanaka N. The expression levels of plasma membrane transporters in the cholestatic liver of patients undergoing biliary drainage and their association with the impairment of biliary secretory function. Am J Gastroenterol 2001; 96:3368-3378.
222. Fromm MF, Kauffmann HM, Fritz P, Burk O, Kroemer HK, Warzok RW, Eichelbaum M, Siegmund W, Schrenk D. The effect of rifampin treatment on intestinal expression of human MRP transporters. Am J Pathol 2000; 157:1575-1580.
223. Laouari D, Yang R, Veau C, Blanke I, Friedlander G. Two apical multidrug transporters, P-gp and MRP2, are differently altered in chronic renal failure. Am J Physiol Renal Physiol 2001; 280:F636-645.
224. König J, Rost D, Cui Y, Keppler D. Characterization of the human multidrug resistance protein isoform MRP3 localized to the basolateral hepatocyte membrane. Hepatology 1999; 29:1156-1163.
225. Xie W, Radominska-Pandya A, Shi Y, Simon CM, Nelson MC, Ong ES, Waxman DJ, Evans RM. An essential role for nuclear receptors SXR/PXR in detoxification of cholestatic bile acids. Proc Natl Acad Sci U S A 2001; 98:3375-3380.
226. Cherrington NJ, Slitt AL, Maher JM, Zhang XX, Zhang J, Huang W, Wan YJ, Moore DD, Klaassen CD. Induction of multidrug resistance protein 3 (mrp3) *in vivo* is independent of constitutive androstane receptor. Drug Metab Dispos 2003; 31:1315-1319.
227. Hitzl M, Klein K, Zanger UM, Fritz P, Nüssler AK, Neuhaus P, Fromm MF. Influence of omeprazole on multidrug resistance protein 3 expression in human liver. J Pharmacol Exp Ther 2003; 304:524-530.
228. Chen C, Klaassen CD. Rat multidrug resistance protein 4 (Mrp4, Abcc4): molecular cloning, organ distribution, postnatal renal expression, and chemical inducibility. Biochem Biophys Res Commun 2004; 317:46-53.
229. Ratajewski M, Bartosz G, Pulaski L. Expression of the human *ABCC6* gene is induced by retinoids through the retinoid X receptor. Biochem Biophys Res Commun 2006; 350:1082-1087.
230. Auyeung DJ, Kessler FK, Ritter JK. Mechanism of rat UDP-glucuronosyltransferase 1A6 induction by oltipraz: evidence for a contribution of the Aryl hydrocarbon receptor pathway. Mol Pharmacol 2003; 63:119-127.
231. Ma Q, Kinneer K, Bi Y, Chan JY, Kan YW. Induction of murine NAD(P)H:quinone oxidoreductase by 2,3,7,8-tetrachlorodibenzo-*p*-dioxin requires the CNC (cap 'n' collar) basic leucine zipper transcription factor Nrf2 (nuclear factor erythroid 2-related factor 2): cross-interaction between AhR (aryl hydrocarbon receptor) and Nrf2 signal transduction. Biochem J 2004; 377:205-213.
232. Chan LM, Lowes S, Hirst BH. The ABCs of drug transport in intestine and liver: efflux proteins limiting drug absorption and bioavailability. Eur J Pharm Sci 2004; 21:25-51.
233. Alrefai WA, Gill RK. Bile acid transporters: structure, function, regulation and pathophysiological implications. Pharm Res 2007; 24:1803-1823.
234. Ballatori N, Christian WV, Lee JY, Dawson PA, Soroka CJ, Boyer JL, Madejczyk MS, Li N. OSTalpha-OSTbeta: a major basolateral bile acid and steroid transporter in human intestinal, renal, and biliary epithelia. Hepatology 2005; 42:1270-1279.
235. Geier A, Wagner M, Dietrich CG, Trauner M. Principles of hepatic organic anion transporter regulation during cholestasis, inflammation and liver regeneration. Biochim Biophys Acta 2007; 1773:283-308.
236. Gerk PM, Vore M. Regulation of expression of the multidrug resistance-associated protein 2 (MRP2) and its role in drug disposition. J Pharmacol Exp Ther 2002; 302:407-415.
237. Zachowski A, Henry JP, Devaux PF. Control of transmembrane lipid asymmetry in chromaffin granules by an ATP-dependent protein. Nature 1989; 340:75-76.
238. Smith AJ, Timmermans-Hereijgers JL, Roelofsen B, Wirtz KW, van Blitterswijk WJ, Smit JJ, Schinkel AH, Borst P. The human MDR3 P-glycoprotein promotes translocation of phosphatidylcholine through the plasma membrane of fibroblasts from transgenic mice. FEBS Lett 1994; 354:263-266.
239. Smit JW, Weert B, Schinkel AH, Meijer DK. Heterologous expression of various P-glycoproteins in polarized epithelial cells induces directional transport of small (type 1) and bulky (type 2) cationic drugs. J Pharmacol Exp Ther 1998; 286:321-327.
240. Matsuzaki Y, Nakano A, Jiang QJ, Pulkkinen L, Uitto J. Tissue-specific expression of the *ABCC6* gene. J Invest Dermatol 2005; 125:900-905.
241. van de Water FM, Masereeuw R, Russel FG. Function and regulation of multidrug resistance proteins (MRPs) in the renal elimination of organic anions. Drug Metab Rev 2005; 37:443-471.
242. Kim WJ, Kakehi Y, Kinoshita H, Arao S, Fukumoto M, Yoshida O. Expression patterns of multidrug-resistance (MDR1), multidrug resistance-associated protein (MRP), glutathione-*S*-transferase-pi (GST-pi) and DNA topoisomerase II (Topo II) genes in renal cell carcinomas and normal kidney. J Urol 1996; 156:506-511.
243. Inui KI, Masuda S, Saito H. Cellular and molecular aspects of drug transport in the kidney. Kidney Int 2000; 58:944-958.
244. Ray AS, Cihlar T, Robinson KL, Tong L, Vela JE, Fuller MD, Wieman LM, Eisenberg EJ, Rhodes GR. Mechanism of active renal tubular efflux of tenofovir. Antimicrob Agents Chemother 2006; 50:3297-3304.
245. Sekine T, Miyazaki H, Endou H. Molecular physiology of renal organic anion transporters. Am J Physiol Renal Physiol 2006; 290:F251-261.
246. Launay-Vacher V, Izzedine H, Karie S, Hulot JS, Baumelou A, Deray G. Renal tubular drug transporters. Nephron Physiol 2006; 103:97-106.
247. Terada T, Inui K. Peptide transporters: structure, function, regulation and application for drug delivery. Curr Drug Metab 2004; 5:85-94.
248. Nishizato Y, Ieiri I, Suzuki H, Kimura M, Kawabata K, Hirota T, Takane H, Irie S, Kusuhara H, Urasaki Y, Urae A, Higuchi S, Otsubo K, Sugiyama Y. Polymorphisms of *OATP-C* (*SLC21A6*) and *OAT3* (*SLC22A8*) genes: consequences for pravastatin pharmacokinetics. Clin Pharmacol Ther 2003; 73:554-565.
249. Jonker JW, Wagenaar E, Van Eijl S, Schinkel AH. Deficiency in the organic cation transporters 1 and 2 (Oct1/Oct2 [Slc22a1/Slc22a2]) in mice abolishes renal secretion of organic cations. Mol Cell Biol 2003; 23:7902-7908.
250. Ganapathy ME, Brandsch M, Prasad PD, Ganapathy V, Leibach FH. Differential recognition of beta -lactam antibiotics by intestinal and renal peptide transporters, PEPT 1 and PEPT 2. J Biol Chem 1995; 270:25672-25677.
251. Jung KY, Takeda M, Shimoda M, Narikawa S, Tojo A, Kim DK, Chairoungdua A, Choi BK, Kusuhara H,

Sugiyama Y, Sekine T, Endou H. Involvement of rat organic anion transporter 3 (rOAT3) in cephaloridine-induced nephrotoxicity: in comparison with rOAT1. Life Sci 2002; 70:1861-1874.
252. Deguchi T, Kusuhara H, Takadate A, Endou H, Otagiri M, Sugiyama Y. Characterization of uremic toxin transport by organic anion transporters in the kidney. Kidney Int 2004; 65:162-174.
253. Yonezawa A, Masuda S, Nishihara K, Yano I, Katsura T, Inui K. Association between tubular toxicity of cisplatin and expression of organic cation transporter rOCT2 (Slc22a2) in the rat. Biochem Pharmacol 2005; 70:1823-1831.
254. Kearney BP, Sayre JR, Flaherty JF, Chen SS, Kaul S, Cheng AK. Drug-drug and drug-food interactions between tenofovir disoproxil fumarate and didanosine. J Clin Pharmacol 2005; 45:1360-1367.
255. Kearney BP, Flaherty JF, Shah J. Tenofovir disoproxil fumarate: clinical pharmacology and pharmacokinetics. Clin Pharmacokinet 2004; 43:595-612.
256. Ray AS, Olson L, Fridland A. Role of purine nucleoside phosphorylase in interactions between 2',3'-dideoxyinosine and allopurinol, ganciclovir, or tenofovir. Antimicrob Agents Chemother 2004; 48:1089-1095.
257. Kotb R, Vincent I, Dulioust A, Peretti D, Taburet AM, Delfraissy JF, Goujard C. Life-threatening interaction between antiretroviral therapy and vinblastine in HIV-associated multicentric Castleman's disease. Eur J Haematol 2006; 76:269-271.
258. Makinson A, Martelli N, Peyrière H, Turriere C, Le Moing V, Reynes J. Profound neutropenia resulting from interaction between antiretroviral therapy and vinblastine in a patient with HIV-associated Hodgkin's disease. Eur J Haematol 2007; 78:358-360.
259. Banks WA. Physiology and pathology of the blood-brain barrier: implications for microbial pathogenesis, drug delivery and neurodegenerative disorders. J Neurovirol 1999; 5:538-555.
260. Löscher W, Potschka H. Blood-brain barrier active efflux transporters: ATP-binding cassette gene family. NeuroRx 2005; 2:86-98.
261. Couture L, Nash JA, Turgeon J. The ATP-binding cassette transporters and their implication in drug disposition: a special look at the heart. Pharmacol Rev 2006; 58:244-258.
262. Girardin F. Membrane transporter proteins: a challenge for CNS drug development. Dialogues Clin Neurosci 2006; 8:311-321.
263. de Boer AG, Gaillard PJ. Drug targeting to the brain. Annu Rev Pharmacol Toxicol 2007; 47:323-355.
264. Dallas S, Miller DS, Bendayan R. Multidrug resistance-associated proteins: expression and function in the central nervous system. Pharmacol Rev 2006; 58:140-161.
265. de Boer AG, van der Sandt IC, Gaillard PJ. The role of drug transporters at the blood-brain barrier. Annu Rev Pharmacol Toxicol 2003; 43:629-656.
266. Hawkins BT, Davis TP. The blood-brain barrier/ neurovascular unit in health and disease. Pharmacol Rev 2005; 57:173-185.
267. Terasaki T, Ohtsuki S. Brain-to-blood transporters for endogenous substrates and xenobiotics at the blood-brain barrier: an overview of biology and methodology. NeuroRx 2005; 2:63-72.
268. Taylor EM. The impact of efflux transporters in the brain on the development of drugs for CNS disorders. Clin Pharmacokinet 2002; 41:81-92.
269. Begley DJ. ABC transporters and the blood-brain barrier. Curr Pharm Des 2004; 10:1295-1312.
270. Deeley RG, Westlake C, Cole SP. Transmembrane transport of endo- and xenobiotics by mammalian ATP-binding cassette multidrug resistance proteins. Physiol Rev 2006; 86:849-899.
271. Siegal T, Zylber-Katz E. Strategies for increasing drug delivery to the brain: focus on brain lymphoma. Clin Pharmacokinet 2002; 41:171-186.
272. Fricker G, Miller DS. Modulation of drug transporters at the blood-brain barrier. Pharmacology 2004; 70:169-176.
273. Zhao Q, Chang XB. Mutation of the aromatic amino acid interacting with adenine moiety of ATP to a polar residue alters the properties of multidrug resistance protein 1. J Biol Chem 2004; 279:48505-48512.
274. Zhang DW, Nunoya K, Vasa M, Gu HM, Theis A, Cole SP, Deeley RG. Transmembrane helix 11 of multidrug resistance protein 1 (MRP1/ABCC1): identification of polar amino acids important for substrate specificity and binding of ATP at nucleotide binding domain 1. Biochemistry 2004; 43:9413-9425.
275. Conseil G, Deeley RG, Cole SP. Polymorphisms of MRP1 (ABCC1) and related ATP-dependent drug transporters. Pharmacogenet Genomics 2005; 15:523-533.
276. Conrad S, Kauffmann HM, Ito K, Leslie EM, Deeley RG, Schrenk D, Cole SP. A naturally occurring mutation in MRP1 results in a selective decrease in organic anion transport and in increased doxorubicin resistance. Pharmacogenetics 2002; 12:321-330.
277. Assaraf YG, Rothem L, Hooijberg JH, Stark M, Ifergan I, Kathmann I, Dijkmans BA, Peters GJ, Jansen G. Loss of multidrug resistance protein 1 expression and folate efflux activity results in a highly concentrative folate transport in human leukemia cells. J Biol Chem 2003; 278:6680-6686.
278. Leslie EM, Létourneau IJ, Deeley RG, Cole SP. Functional and structural consequences of cysteine substitutions in the NH_2 proximal region of the human multidrug resistance protein 1 (MRP1/ABCC1). Biochemistry 2003; 42:5214-5224.
279. Ito K, Oleschuk CJ, Westlake C, Vasa MZ, Deeley RG, Cole SP. Mutation of Trp^{1254} in the multispecific organic anion transporter, multidrug resistance protein 2 (MRP2) (ABCC2), alters substrate specificity and results in loss of methotrexate transport activity. J Biol Chem 2001; 276:38108-38114.
280. Büchler M, König J, Brom M, Kartenbeck J, Spring H, Horie T, Keppler D. cDNA cloning of the hepatocyte canalicular isoform of the multidrug resistance protein, cMRP, reveals a novel conjungate export pump deficient in hyperbilirubinemic rats. J Biol Chem 1996; 271:15091-15098.
281. Ito K, Suzuki H, Hirohashi T, Kume K, Shimizu T, Sugiyama Y. Molecular cloning of canalicular multispecific organic anion transporter defective in EHBR. Am J Physiol 1997; 272(1 Pt 1):G16-22.
282. Paulusma CC, Bosma PJ, Zaman GJ, Bakker CT, Otter M, Scheffer GL, Scheper RJ, Borst P, Oude Elferink RP. Congenital jaundice in rats with a mutation in a multidrug resistance-associated protein gene. Science 1996; 271:1126-1128.
283. Kartenbeck J, Leuschner U, Mayer R, Keppler D. Absence of the canalicular isoform of the *MRP* gene-encoded conjugate export pump from the hepatocytes in Dubin-Johnson syndrome. Hepatology 1996; 23:1061-1066.
284. Machida I, Inagaki Y, Suzuki S, Hayashi H, Wakusawa S.

Mutation analysis of the multidrug resistance protein 2 (*MRP2*) gene in a Japanese patient with Dubin-Johnson syndrome. Hepatol Res 2004; 30:86-90.
285. Wakusawa S, Machida I, Suzuki S, Hayashi H, Yano M, Yoshioka K. Identification of a novel 2026G→C mutation of the *MRP2* gene in a Japanese patient with Dubin-Johnson syndrome. J Hum Genet 2003; 48:425-429.
286. Hirouchi M, Suzuki H, Itoda M, Ozawa S, Sawada J, Ieiri I, Ohtsubo K, Sugiyama Y. Characterization of the cellular localization, expression level, and function of SNP variants of MRP2/ABCC2. Pharm Res 2004; 21:742-748.
287. Ito S, Ieiri I, Tanabe M, Suzuki A, Higuchi S, Otsubo K. Polymorphism of the ABC transporter genes, *MDR1*, *MRP1* and *MRP2/cMOAT*, in healthy Japanese subjects. Pharmacogenetics 2001; 11:175-184.
288. Mor-Cohen R, Zivelin A, Rosenberg N, Shani M, Muallem S, Seligsohn U. Identification and functional analysis of two novel mutations in the multidrug resistance protein 2 gene in Israeli patients with Dubin-Johnson syndrome. J Biol Chem 2001; 276:36923-36930.
289. Izzedine H, Hulot JS, Villard E, Goyenvalle C, Dominguez S, Ghosn J, Valantin MA, Lechat P, Deray AG. Association between *ABCC2* gene haplotypes and tenofovir-induced proximal tubulopathy. J Infect Dis 2006; 194:1481-1491.
290. Lang T, Hitzl M, Burk O, Mornhinweg E, Keil A, Kerb R, Klein K, Zanger UM, Eichelbaum M, Fromm MF. Genetic polymorphisms in the multidrug resistance-associated protein 3 (*ABCC3, MRP3*) gene and relationship to its mRNA and protein expression in human liver. Pharmacogenetics 2004; 14:155-164.
291. Le Saux O, Urban Z, Tschuch C, Csiszar K, Bacchelli B, Quaglino D, Pasquali-Ronchetti I, Pope FM, Richards A, Terry S, Bercovitch L, de Paepe A, Boyd CD. Mutations in a gene encoding an ABC transporter cause pseudoxanthoma elasticum. Nat Genet 2000; 25:223-227.
292. Ringpfeil F, Lebwohl MG, Christiano AM, Uitto J. Pseudoxanthoma elasticum: mutations in the *MRP6* gene encoding a transmembrane ATP-binding cassette (ABC) transporter. Proc Natl Acad Sci U S A 2000; 97:6001-6006.
293. Uitto J. Pseudoxanthoma elasticum-a connective tissue disease or a metabolic disorder at the genome/environment interface? J Invest Dermatol 2004; 122: ix-x.
294. Hu X, Plomp AS, van Soest S, Wijnholds J, de Jong PT, Bergen AA. Pseudoxanthoma elasticum: a clinical, histopathological, and molecular update. Surv Ophthalmol 2003; 48:424-438.
295. Gheduzzi D, Guidetti R, Anzivino C, Tarugi P, Di Leo E, Quaglino D, Ronchetti IP. ABCC6 mutations in Italian families affected by pseudoxanthoma elasticum (PXE). Hum Mutat 2004; 24:438-439.
296. Chassaing N, Martin L, Mazereeuw J, Barrié L, Nizard S, Bonafé JL, Calvas P, Hovnanian A. Novel ABCC6 mutations in pseudoxanthoma elasticum. J Invest Dermatol 2004; 122:608-613.
297. Hu X, Peek R, Plomp A, ten Brink J, Scheffer G, van Soest S, Leys A, de Jong PT, Bergen AA. Analysis of the frequent R1141X mutation in the *ABCC6* gene in pseudoxanthoma elasticum. Invest Ophthalmol Vis Sci 2003; 44:1824-1829.
298. Hendig D, Schulz V, Eichgrün J, Szliska C, Götting C, Kleesiek K. New *ABCC6* gene mutations in German pseudoxanthoma elasticum patients. J Mol Med 2005; 83:140-147.
299. Germain DP, Remones V, Perdu J, Jeunemaitre X. Identification of two polymorphisms (c189G>C; c190T>C) in exon 2 of the human *MRP6* gene (ABCC6) by screening of Pseudoxanthoma elasticum patients: possible sequence correction? Hum Mutat 2000; 16:449.
300. Gottesman MM, Fojo T, Bates SE. Multidrug resistance in cancer: role of ATP-dependent transporters. Nat Rev Cancer 2002; 2:48-58.
301. Teodori E, Dei S, Martelli C, Scapecchi S, Gualtieri F. The functions and structure of ABC transporters: implications for the design of new inhibitors of Pgp and MRP1 to control multidrug resistance (MDR). Curr Drug Targets 2006; 7:893-909.
302. Sauna ZE, Peng XH, Nandigama K, Tekle S, Ambudkar SV. The molecular basis of the action of disulfiram as a modulator of the multidrug resistance-linked ATP binding cassette transporters MDR1 (ABCB1) and MRP1 (ABCC1). Mol Pharmacol 2004; 65:675-684.
303. Perego P, De Cesare M, De Isabella P, Carenini N, Beggiolin G, Pezzoni G, Palumbo M, Tartaglia L, Pratesi G, Pisano C, Carminati P, Scheffer GL, Zunino F. A novel 7-modified camptothecin analog overcomes breast cancer resistance protein-associated resistance in a mitoxantrone-selected colon carcinoma cell line. Cancer Res 2001; 61:6034-6037.
304. Vail DM, Amantea MA, Colbern GT, Martin FJ, Hilger RA, Working PK. Pegylated liposomal doxorubicin: proof of principle using preclinical animal models and pharmacokinetic studies. Semin Oncol 2004; 31:16-35.
305. Cascorbi I. Role of pharmacogenetics of ATP-binding cassette transporters in the pharmacokinetics of drugs. Pharmacol Ther 2006; 112:457-473.
306. Szakács G, Paterson JK, Ludwig JA, Booth-Genthe C, Gottesman MM. Targeting multidrug resistance in cancer. Nat Rev Drug Discov 2006; 5:219-234.
307. Conrad S, Kauffmann HM, Ito K, Deeley RG, Cole SP, Schrenk D. Identification of human multidrug resistance protein 1 (MRP1) mutations and characterization of a G671V substitution. J Hum Genet 2001; 46:656-663.
308. Cai L, Lumsden A, Guenther UP, Neldner SA, Zäch S, Knoblauch H, Ramesar R, Hohl D, Callen DF, Neldner KH, Lindpaintner K, Richards RI, Struk B. A novel Q378X mutation exists in the transmembrane transporter protein ABCC6 and its pseudogene: implications for mutation analysis in pseudoxanthoma elasticum. J Mol Med 2001; 79:536-546.
309. Pulkkinen L, Nakano A, Ringpfeil F, Uitto J. Identification of ABCC6 pseudogenes on human chromosome 16p: implications for mutation detection in pseudoxanthoma elasticum. Hum Genet 2001; 109:356-365.
310. Meloni I, Rubegni P, De Aloe G, Bruttini M, Pianigiani E, Cusano R, Seri M, Mondillo S, Federico A, Bardelli AM, Andreassi L, Fimiani M, Renieri A. Pseudoxanthoma elasticum: Point mutations in the *ABCC6* gene and a large deletion including also ABCC1 and MYH11. Hum Mutat 2001; 18:85.

(*Received November 24, 2008; Accepted December 7, 2008*)