Pflugers Arch - Eur J Physiol (2007) 453:735–744
DOI 10.1007/s00424-006-0134-x

INVITED REVIEW

ATP-binding cassette, subfamily G (ABCG family)

Hiroyuki Kusuhara · Yuichi Sugiyama

Received: 19 May 2006 / Accepted: 3 July 2006 / Published online: 16 September 2006

Abstract This review summarizes the characteristics of the ATP-binding cassette, subfamily G (ABCG family), which has five members: ABCG1, ABCG2, ABCG4, ABCG5, and ABCG8. The members consist of a single ABC cassette in the amino terminal followed by six putative transmembrane domains, and to become functionally active, they form homo- or obligate heterodimers. Except for ABCG2, the members of the ABCG family play an important role in efflux transport of cholesterol. Mutations causing a loss of function of ABCG5 or ABCG8 are associated with sitosterolemia characterized by accumulation of phyto- and shellfish sterols. Unlike other members, ABCG2 is not involved in cholesterol efflux, but it exhibits broad substrate specificity to xenobiotic compounds. ABCG2 confers cancer cells resistance to anticancer drugs and plays a critical role in the pharmacokinetics of drugs in the clearance organs and tissue barriers. ABCG2 is also associated with a subpopulation phenotype of stem cells. Genetic polymorphisms of ABCG2 have been suggested to account for the interindividual differences in the pharmacokinetics of drugs.

Keywords Cholesterol · High-density lipoprotein · Sitosterolemia · Efflux · Multidrug resistance · Drug disposition

H. Kusuhara (✉) · Y. Sugiyama
Graduate School of Pharmaceutical Sciences,
The University of Tokyo,
7-3-1 Hongo,
Bunkyo-ku, Tokyo 113-0033, Japan
e-mail: kusuhara@mol.f.u-tokyo.ac.jp

Y. Sugiyama
e-mail: sugiyama@mol.f.u-tokyo.ac.jp

Introduction

The ATP-binding cassette subfamily G (ABCG) consists of five members: ABCG1, ABCG2, ABCG4, ABCG5, and ABCG8 (Table 1). Among the members, ABCG1 and ABCG4 exhibit high identity (70%) at the amino acid level, whereas others exhibit low identity (at most, 30%) (Fig. 1). They are mammalian homologs of the Drosophila gene White, which forms obligate heterodimers with Scarlet or Brown and plays an essential role in the cellular uptake of precursors of the eye pigments [18, 71, 73]. Consistent with the Drosophila homolog, the members of the ABCG family consist of a single ABC cassette in the amino terminal followed by six putative transmembrane domains, and thus, they are referred to as half-sized ABC transporters. To become functionally active, they form a homodimer (ABCG1, ABCG2, and ABCG4) or an obligate heterodimer (ABCG5 and ABCG8). The members of ABCG family, except ABCG2, play significant role in the efflux transport of cholesterol (Fig. 2). They facilitate the efflux of excess cholesterol to high-density lipoprotein (HDL), a key player in the reverse cholesterol transport from macrophage to the liver (ABCG1 and ABCG4), and mediate the biliary excretion of cholesterol (ABCG5 and ABCG8). In addition, ABCG5 and ABCG8 also mediate the biliary excretion of phyto- and shellfish sterols, and intestinal efflux to prevent their accumulation. Unlike other members, ABCG2 accepts a variety of structurally unrelated compounds as substrate and plays important roles in the cancer chemotherapy and drug disposition in the clearance organs (liver and kidney), site of absorption (intestine), and tissue barriers (blood–brain barrier and placenta) (Fig. 2).

The present manuscript concentrates on the molecular characteristics of members of the ABCG family and presents the latest findings. Other review articles are

Table 1 Members of ABCG family

Gene name	Aliases	Substrates	Inhibitors	Tissue distribution and cellular/subcellular expression	Link to disease (OMIM ID)	Gene locus	OMIM
ABCG1	ABC8	Cholesterol		Ubiquitous high: adrenal gland, lung, heart, spleen		21q22.2–q22.3	603076
ABCG2	BCRP, MXR, ABCP	Very broad	Ko143, GF120918, fumitremorgin C, novobiocin, imatinib	Ubiquitous high: placenta, plasma membrane (apical)		4q22	603756
ABCG4		Cholesterol		Brain, macrophage plasma membrane		11q23.3	607784
ABCG5	Sterolin-1	Cholesterol		Liver, intestine	Sitosterolemia (OMIM: 210250)	2p21	605459
ABCG8	Sterolin-2	Phytosterols, shellfish sterols		Plasma membrane (apical)		2p21	605460

available elsewhere on ABCG1 [67], ABCG2 [3, 16, 39, 74], and ABCG5/ABCG8 [12, 23, 83].

ABCG1 (ABC8)

ABCG1 was cloned from the mouse macrophage cell line P388D1 [65] and from the human retina and fetal brain and the Jurkat T-cell line, which exhibits 34% identity to Drosophila White at the amino acid level [13, 14]. The cDNAs cloned by Chen et al. [13] and Croop et al. [14] encode 674 and 638 amino acid proteins, respectively, and the cDNA cloned by Croop et al. [14] lacks 36 amino acids at the amino terminal. Subsequent analyses have shown that other variants are produced by alternative splicing (see regulation and genomic structure of ABCG1) [32, 47, 58]. Northern blot analysis showed abundant expression of ABCG1 in the spleen, brain, and lung [14], and dot blot analysis revealed ubiquitous expression, with the highest expression in the adrenal glands, lung, heart, and spleen [35]. The calculated molecular mass of ABCG1 encoded by cDNA cloned by Croop et al. [14] is ~70 kDa, and transfection of the cDNA yielded a 60-kDa protein in baby hamster kidney (BHK) cells, and that cloned by Chen et al. [13] yielded protein with a greater molecular mass [77]. Two different apparent molecular masses of ABCG1 have been reported in human macrophages: 60 kDa [77] and 110 kDa [35].

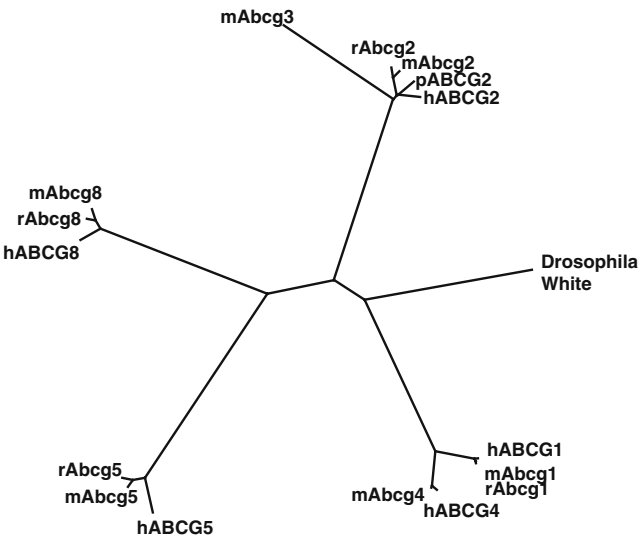

Fig. 1 Phylogenetic tree of ABG family and Drosophila White. ClustalW was used to align full-length ABCG1, ABCG2, Abcg3, ABCG4, ABCG5, ABCG8, and Drosophila White amino acid sequences, which were subjected to phylogenetic analysis using ClustalW. Accession numbers: ABCG1 (human, P45844; rat, NP_445954; mouse, NP_033723), ABCG2 (human, AAP44087; porcine, NP_999175; rat, NP_852046; mouse, NP_036050), Abcg3 (mouse, NP_084515), ABCG4 (human, NP_071452; mouse, NP_620405), ABCG5 (human, NP_071881; rat, NM_053754; mouse, NM_031884), ABCG8 (human, NM_022437; rat, NM_130414; mouse, NM_026180), and Drosophila White (AAS09823)

Light microscopic immunocytochemical analysis showed that the ABCG1-specific signals were in the intracellular compartment of the small rounded-off macrophages treated with acetylated low-density lipoprotein (LDL) for 2 days, and faint signals were detected in the perinuclear compartment of the spread-out macrophages [35]. Flow cytometric analysis using an antihuman ABCG1 antiserum, which recognizes the extracellular loop between the putative transmembrane domains 5 and 6, demonstrated the plasma membrane expression of ABCG1 in macrophages [35], and biotinylation of cell surface proteins yielded biotinylated ABCG1 in ABCG1 exogenously expressed in BHK cells, supporting the plasma membrane expression of ABCG1 [77]. Treatment with a non-cell-membrane-permeable cross-linking reagent, disuccinimidyl suberate, gave bands with an apparent molecular weight of dimeric or trimeric ABCG1 in ABCG1 exogenously expressed BHK cells, suggesting that ABCG1 forms a homodimer or trimer to function as an efflux transporter in the plasma membrane [77].

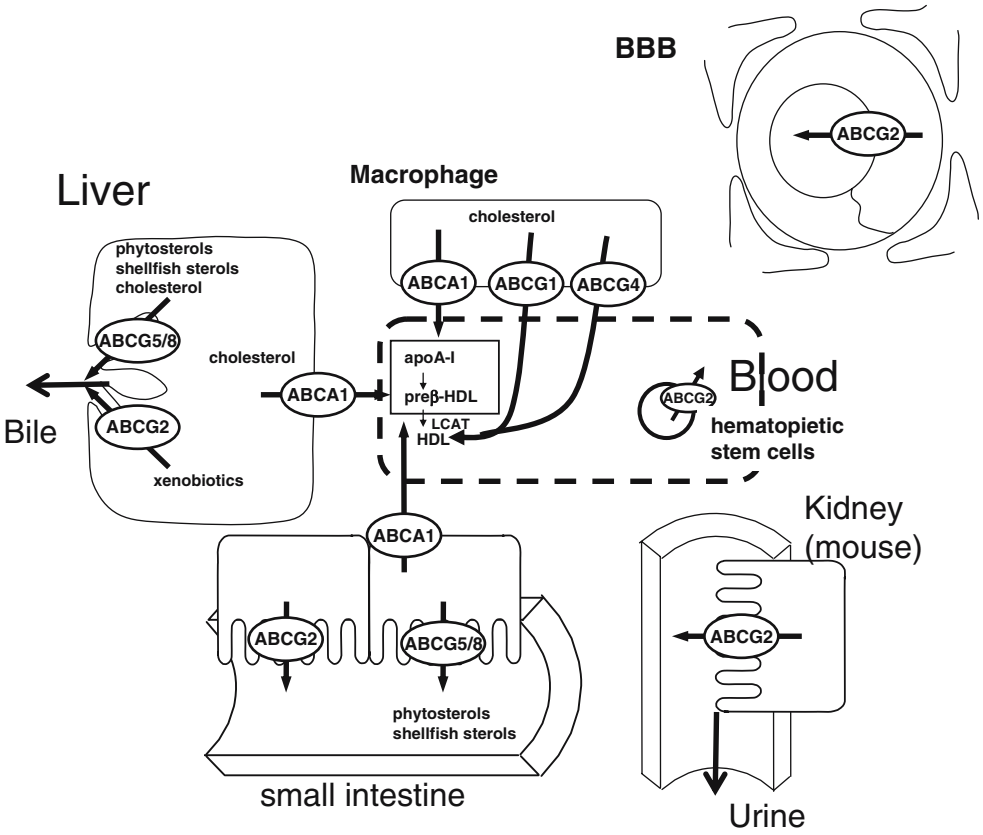

Fig. 2 Schematic diagram of expression site of the members of ABCG family. ABCG1 and ABCG4 can transfer cholesterol to mature HDL, but not to apo-AI in macrophage. ABCG5 and ABCG8, forming obligatory heterodimer, mediate the biliary and intestinal efflux of cholesterol, and phyto- and shellfish sterols, and impairment of ABCG5/8 activity is associated with sitosterolemia. ABCG2 mediates efflux of xenobiotics, but not cholesterol, in the intestine, liver (biliary excretion), and rodent kidney (tubular secretion). In the tissue barriers, such as blood–brain barrier and placenta, ABCG2 acts a barrier to prevent the distribution of xenobiotics to the brain and fetus. ABCG2 is associated with side population phenotype of hematopoietic stem cells, characterized by low accumulation of a dye Hoechst 33342. *BBB* blood–brain barrier, *HDL* high-density lipoprotein, *LCAT* lecithin:cholesterol acyltransferase

ABCG1 plays a significant role in the efflux transport of excess cholesterol to HDL in macrophages. Knockdown of the ABCG1 by RNA interference caused a significant reduction in the cholesterol efflux to the HDL from macrophages under the condition that the ABCG1 expression was induced by treatment with acetylated LDL or a synthetic agonist of liver X receptor (LXR/ NR1H3), T0901317 [35, 82]. The efflux transport of cholesterol to HDL by ABCG1 has also been characterized in cDNA transfectants [77, 82]. Transfection of ABCG1 cDNA to a human embryo kidney cell line (HEK293) increased cholesterol efflux to both smaller (HDL-3, density 1.125–1.210 g/ml) and larger (HDL-2, density 1.063–1.125 g/ml) subclasses and, to a lesser degree, to LDL, but not to apoA-I, a major apolipoprotein of HDL, to which ABCA1 mediates the efflux of cellular cholesterol [82]. In turn, ABCA1 makes only a limited contribution to the cholesterol efflux to HDL in macrophage since the efflux was unchanged in Abca1 knockout mice [82]. Exogenous expression of ABCG1 induces a redistribution of cholesterol, increasing the cell-surface pool of cholesterol for removal by HDL, and the fraction of esterified cholesterol in BHK cells, presumably increasing the transport of cholesterol to the endoplasmic reticulum, where it is esterified by acyl-CoA: cholesterol acyltransferase [77].

Abcg1 knockout mice have been developed by Kennedy et al. [33], and these exhibit massive accumulation of cholesterol and neutral lipids in the macrophages and liver when fed a high-fat and high-cholesterol diet, whereas there was no difference when the animals were fed a normal diet [33]. The efflux of cholesterol to HDL was apparently not significantly different in the macrophages from wild-type and Abcg1 knockout mice; however, when the macrophages were treated with LXR ligand (T0901317), the efflux transport of cholesterol to HDL was increased in wild-type mice, but unchanged in Abcg1 knockout mice supporting the role of Abcg1 in the efflux of excess cholesterol [33].

Regulation and genomic structure of ABCG1

ABCG1 expression is regulated by the cellular cholesterol content. It is increased following treatment with acetylated LDL (cholesterol-loading condition), and downregulated following treatment with HDL-3 at both mRNA and protein levels in monocyte-derived macrophages [35]. Nuclear receptors, such as the LXR and peroxisome proliferative-activated receptor (PPAR), have been suggested to regulate the expression of ABCG1. Endogenous LXR agonists, such as 22(*S*)-hydroxycholesterol and 22(*R*)-hydroxycholesterol, induce ABCG1 expression in THP1 macrophages, and this increase is abrogated in LXRα/β double knockout animals

[41]. In addition, PPARγ positively regulates ABCG1 in macrophages in an LXR-independent manner [2, 45].

The genomic organization of human ABCG1 was initially reported to consist of 15 exons [42]. The transcript reported by Chen et al. [13] starts from Exon 1, and that by Croop et al. [14] starts from Exon 2. However, subsequent analysis by 5′-RACE using RNA from THP1 cells treated with LXRα and RXRα ligands suggested the possibility of alternative splicing and demonstrated an additional one and four exons downstream and upstream of the initially identified Exon 1, respectively [47]. Lorkowski et al. identified six variants with different amino acids at the amino terminal, including one reported by Chen et al. [13]. Furthermore, Kennedy et al. found an additional three exons between Exon 7 and 8, and the ABCG1 gene consists of 23 exons [32]. Alternative splicing produces seven ABCG1 variants: (i) Exon 1, 2, and 7, (ii) Exon 1, 2, 3, and 7, (iii) Exon 4 and 7, (iv) Exon 5 and 7, (v) Exon 6 and 7, and (vi) Exon 8–10, with common exons (Exon 11–23) in THP1 cells treated by 20(*S*)-hydroxycholesterol to induce ABCG1 expression [32, 47]. Northern blot analysis confirmed that the variant consisting of Exon 8–23 and the variants including Exon 7 were induced in the cells, although the abundance of each variant remains unknown [32]. Since the ABCG1 cloned by Chen et al. [13] and that cloned by Croop et al. [14] have functional activity [77], variations at the amino terminal may not be essential for the transport activity.

Putative promoters have been hypothesized upstream of Exon 1, 4, 5, 6, and 8 [32, 42, 47]. Among them, the promoter located upstream of Exon 1, 4, 5, and 6 is TATAless [42, 47], whereas that upstream of Exon 8 contains a TATA box [32]. Two functional LXR responsive elements have been identified only upstream of Exon 8 [32]. A putative PPAR/RXR binding motif lies upstream of Exon 1, and a putative sterol response element and two RXR binding motifs have been suggested upstream of Exon 5 [47].

ABCG2 (BCRP/ABCP/MXR)

ABCG2 was initially identified as an overexpressed protein in MCF-7/AdrVp, a cell line obtained by continuous exposure to doxorubicin and verapamil, and does not express P-glycoprotein (P-gp) and multidrug resistance associated protein [17]. In addition to tumor cells, ABCG2 is highly expressed in the placenta, followed by the brain, liver, prostate, small intestine, and colon at the mRNA level [6, 17]. Immunohistochemical staining showed apical localization in normal tissues, such as the colon epithelium, placenta syncytiotrophoblast, small intestine epithelium, liver (bile canaliculi), mammary gland (lobules and lactiferous ducts) and vein endothelium and capillaries [50], and mouse renal tubules [29].

ABCG2 forms a homodimer linked by a disulfide bond in the plasma membrane since ABCG2 migrates as a dimer without reducing agents on sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) [31, 54], and two differentially tagged forms of ABCG2 underwent co-immunoprecipitation [31]. ABCG2 has three preserved cysteine residues (592, 603, and 608) in the large extracellular loop between putative transmembrane domain 5 and 6. Among these residues, cysteine 603 is involved in the formation of an intermolecular disulfide bond, whereas the others have been suggested to form an intramolecular disulfide bond [24]. Dimerization is not essential for membrane localization and transport activity since treatment with mercaptoethanol, a reducing agent, did not affect the ATP-dependent transport of methotrexate by ABCG2 [54]. Furthermore, substitution of alanine for cysteine 603 did not affect membrane localization and resistance to mitoxantrone, although it abolished dimerization [24]. It should be noted that, recently, higher forms of oligomers (tetramer and dodecamer) of ABCG2 have been suggested, in which a homodimer linked by a disulfide bond is the minimum unit [84].

Unlike ABCG1 and ABCG4, ABCG2 does not mediate the efflux of cholesterol to HDL [82], but it plays physiologically and pharmacologically important roles. ABCG2 confers resistance to anticancer drugs, such as mitoxantrone, doxorubicin, and daunorubicin, by decreasing their cellular accumulation, and thus, ABCG2 has been referred to as breast cancer resistant protein (BCRP) [17]. Subsequent analyses have shown that overexpression of ABCG2 results in the acquisition of resistance to anticancer drugs, such as camptothecin derivatives (topotecan [4], SN-38 [49], 9-aminocamptothecin [49]), antifolates (methotrexate and its polyglutamate form, GW1843 and tomudex) [68, 79, 78], flavopiridol [64], and bisantrene [46]. Attention should be paid to the acquired mutations of ABCG2 in some tumor cell lines (R482G and R482T), which have been shown to alter the spectrum of resistance to anticancer drugs. Cells expressing these mutants exhibit higher resistance to anthracylcines [26], but weak or minimal resistance to methotrexate [80]. The mutants acquire the ability to carry out efflux transport of anthracylcines and rhodamine 123 [26], but lose the ability to transport methotrexate [54]. In addition to anticancer drugs, cumulative studies have demonstrated the broad substrate specificity of ABCG2, and it accepts a variety of drugs [10, 25, 30, 52, 51, 86], endogenous compounds, sulfate conjugates [1, 56, 72], and carcinogens [75, 76] as substrate. For investigating functional activity of ABCG2, potent inhibitors of BCRP have been reported, which

include fumitremorgin C [61] and its derivative Ko143 [5], GF120918 [4], novobiocin [69], and imatinib (Gleevec) [59]. It should be noted that GF120918 is also a potent inhibitor of P-gp, whereas Ko143 shows almost 300-fold more inhibition potency to BCRP than P-gp [5].

The functional importance of ABCG2 in the normal tissues has been investigated using Abcg2 knockout mice. Jonker et al. [29] and Zhou et al. [91] have developed Abcg2 knockout mice independently. Abcg2 knockout mice exhibit a higher concentration of unconjugated bilirubin in plasma, and a heme precursor, protoporphyrin IX, accumulates in the plasma and erythrocytes [29]. Furthermore, feeding Abcg2 knockout mice with a diet containing alfalfa (*Medicago sativa*) leaf concentrate resulted in lethal phototoxic lesions on light-exposed skin of these animals due to accumulation of pheophorbide a (a phototoxic porphyrin catabolite of chlorophyll) [29]. Abcg2 knockout mice are 100-fold more sensitive to pheophorbide-a-induced phototoxicity than wild-type mice, and accumulation of pheophorbide a in bone-marrow-derived cells is likely associated with photosensitivity since bone-marrow transplantation from wild-type mice to the knockout mice results in the attenuation of the toxicity.

For drugs, it has also been shown that BCRP limits the oral absorption of topotecan [29], salfasalzine [86], and ciprofloxacin [52]. Abcg2 has been shown to account for the efflux of intracellularly formed glucuronide and sulfate conjugates (E3040 glucuronide, E3040 sulfate, and 4-methylumbelliferone sulfate) [1]; the active form of the ester-type prodrug of ME3277 [38] in the small intestine; the biliary excretion of drugs such as nitrofurantoin [51], methotrexate [10], pitavastatin [25], and salfasalazine [86]; and the urinary excretion of methotrexate [10] and E3040 sulfate [56]. As far as the blood–brain barrier is concerned, the importance of BCRP remains controversial. In contrast to in vitro data supporting the functional importance of ABCG2 in the blood–brain barrier [88], imatinib is the only compound exhibiting an increased brain concentration in Abcg2 knockout mice [11]. No change has been reported for other BCRP substrates, such as mitoxantrone [44], dehydroepiandrosterone sulfate (DHEAS) [44], and PhIP [75]. In the placenta, ABCG2 prevents the fetal accumulation of topotecan, and the ratio of topotecan fetal to maternal plasma concentrations is increased in Abcg2 knockout mice [29]. ABCG2 expression is highly induced in the apical membrane of alveolar epithelial cells during late pregnancy and especially during lactation [30], and is involved in the secretion of drugs into milk. The milk-to-plasma concentration ratio of topotecan [30], PhIP [30], acyclovir [30], nitrofurantoin [51], and ciprofloxacin [52] was markedly reduced in Abcg2 knockout mice, but not that of DHEAS and folate [30].

ABCG2 is also associated with the side population phenotype, a phenotype of stem cells displaying low Hoechst 33342 accumulation [66, 90]. The low cellular accumulation of Hoechst 33342 is ascribed to the ABCG2-mediated efflux since the number of cells displaying such phenotype was reduced in Abcg2 knockout mice. Knockout of Abcg2 does not affect differentiation of bone marrow cells [29], whereas overexpression of ABCG2 in bone marrow cells has been suggested to inhibit hematopoiesis [90].

Regulation and genomic organization of ABCG2

The ABCG2 gene consists of 16 exons, and a putative TATAless promoter has been suggested upstream of Exon 1 [8], where the binding motifs of estrogen receptor (ERα) and hypoxia-inducible factor 1 (HIF1) are located and where the binding of ERα and HIF1 was confirmed by electrophoretic mobility shift analysis [20, 40]. Although the responsive element has not been identified, aryl hydrocarbon receptor (AhR) and PPAR have been suggested to upregulate BCRP expression [19, 57].

The effect of estradiol on the ABCG2 expression appears cell-dependent and controversial. ABCG2 mRNA was upregulated by estradiol in ERα-positive T47D:A18 breast cancer cells; however, estrogens (estradiol and estrone) and diethylstilbestrol (ER agonist) reduced the protein expression of ABCG2 in an ERα-dependent manner without affecting the mRNA expression in MCF-7 [28]. Consistent with the reduced ABCG2 expression, the cellular accumulation of topotecan was increased by the treatment of estrogen in MCF7 cells [28]. It was suggested that ERα inhibits the translation of ABCG2 in MCF7 cells [28]. In human placental BeWo cells, estradiol reduced both BCRP mRNA and protein, which were restored by an ER antagonist [81]. In contrast to estrogens, progesterone upregulated both BCRP mRNA and protein via a progesterone receptor independent pathway in BeWo cells, and estradiol enhanced the effect of progesterone in an ER-dependent manner [81].

Hypoxia also induces ABCG2 expression through the binding of HIF1 to the hypoxia response element of the ABCG2 gene to prevent cellular accumulation of heme or porphyrin accumulation in progenitor cells [40]. Treatment of Caco2 cells with AhR agonists resulted in a significant increase in BCRP expression which was inhibited by AhR antagonist PD98059 [19]. Repeated administration of clofibrate, a PPARα agonist, for 10 days significantly increased hepatic expression of BCRP in mice. PPARα mediates such induction since the induction by clofibrate was not observed in PPARα knockout mice.

Polymorphisms of ABCG2

Nonsynonymous single nucleotide polymorphisms have been reported. Among them, C421A, a frequent polymorphism in the Japanese population, substituting Lys for Gln, has been associated with reduced protein expression of ABCG2 compared with wild-type ABCG2 without affecting the mRNA expression in murine fibroblast PA317 cells, and the cell line expressing C421A ABCG2 exhibits reduced cellular accumulation of topotecan, resulting in weak drug resistance to SN-38, mitoxantrone, and topotecan [27]. Similar results were obtained in HEK293 cells infected with adenovirus harboring ABCG2 cDNA, and introduction of the polymorphism caused reduced ATP-dependent uptake of ABCG2 substrates by membrane vesicles in proportion to the reduced protein expression [37]. In contrast, the protein expression of C421A ABCG2 was unchanged in LLC-PK1, although increased sensitivity to anticancer drugs and cellular accumulation of these drugs were observed in comparison with wild-type ABCG2 [55]. In this study, the ATPase activity of C421A ABCG2 was significantly reduced compared with that of wild-type ABCG2. In normal tissue, C421A is associated with reduced ABCG2 protein expression in the placenta [36], but not in the small intestine [87]. Controversial results were obtained in the case of another frequent polymorphism, G34A, substituting Met for Val. Introduction of this polymorphism resulted in poor apical membrane distribution in LLC-PK1 cells, leading to greater cellular accumulation compared with cells expressing wild-type ABCG2 [55], whereas it had no effect when expressed in the murine cell line, Sf9, and HEK293 cells [27, 37, 55].

Clinical studies focused on C421A have been carried out. Heterozygotes exhibited significantly higher plasma concentrations of diflomotecan after intravenous administration, whereas the plasma concentration–time profiles were similar after oral administration [70]. Such a discrepancy cannot be explained from a pharmacokinetic viewpoint, and the effect of such a polymorphism on the plasma concentration should be theoretically clearer after oral administration. In another report, the area under the curve of the plasma concentration of rosuvastatin following a single oral administration was greater in the hetero- and homozygotes [89], but heterozygotes did not show any difference in the pharmacokinetic profile of irinotecan and its active metabolite SN-38 [15].

ABCG3

Abcg3 was identified by a computer search of the mouse EST databases [53]. Abcg3 consists of 650 amino acids, and reverse transcriptase polymerase chain reaction (RT-PCR) analysis showed that it was abundantly expressed in the thymus and spleen. The functions of Abcg3 remain unknown. However, since Abcg3 contains unusual residues in the Walker A motif and signature (C) domains, it is questionable whether Abcg3 exhibits ATPase activity and acts as an efflux pump [53].

ABCG4

ABCG4 was identified as the second most strongly induced member of the ABCG family in human monocyte-derived macrophages treated with 9-*cis*-retinoic acid and 22(*R*)-hydroxycholesterol, agonists of RXR and LXR, respectively [21]. Northern blot analysis revealed that ABCG4 is highly expressed in the brain, where ABCG4 is widely distributed other than the spinal chord, followed by the heart, and a longer transcript was detected in the thymus, whereas the transcript of its mouse homolog is highly expressed in the brain and spleen [7]. The ABCG4 gene consists of at least 14 exons, although Engel et al. suggested an additional exon upstream of the current Exon 1 since there are no transcription initiation elements upstream of Exon 1 [21].

Wang et al. showed that ABCG4 also mediates the efflux of cellular cholesterol to HDL [82]. As in the case of ABCG1, transfection of ABCG4 cDNA to HEK293 increased the cholesterol efflux to both smaller (HDL-3, density 1.125–1.210 g/ml) and larger (HDL-2, density 1.063–1.125 g/ml) subclasses and, to a lesser degree, to LDL, but not to apoA-I. The fact that the efflux of cholesterol to HDL was almost unchanged in macrophage of Abcg1 knockout mice following treatment of LXR and RXR agonists [33] may suggest its minor contribution, at least in rodent.

ABCG5/ABCG8

Human ABCG5 was initially identified as the human counterpart of the rodent isoform that was induced in the liver and small intestine by treatment with an agonist of LXR, T0901317 [9]. Subsequently, human ABCG8 was cloned as a homolog, the gene of which is adjacent to the ABCG5 gene [9]. The ABCG5 and ABCG8 are closely arranged with an insertion of 85 or 140 bases in opposite orientation [9, 62]. Independently, Lee et al. [43] identified ABCG5 by positional cloning as the gene expressed in the liver and small intestine which has been mapped to the locus associated with sitosterolemia [Online Mendelian Inheritance in Man (OMIM) 210250, see below]. Northern blot analysis showed that the expression of ABCG5 and ABCG8 overlapped in the liver and small intestine and, to a

lesser extent, in the colon, where both ABCG5 and ABCG8 are expressed at the apical membrane [22]. Coexpression of ABCG5 and ABCG8 is essential for maturation [22]. When ABCG5 and ABCG8 are independently expressed, they are restricted to the endoplasmic reticulum, and coexpression results in the translocation of their proteins to the plasma membrane. The heteromeric dimerization of ABCG5 and ABCG8 was confirmed by immunoprecipitation [22]. Differentially tagged ABCG8 and ABCG5 were coprecipitated when they were coexpressed in CHO-K1 cells, whereas when expressed independently, they were recovered from the supernatant fraction.

Regulation of ABCG5 and ABCG8

As described previously, ABCG5 was initially identified as the gene positively regulated by LXR [9]. Indeed, in mice fed a high-cholesterol (2%) diet or a high-cholesterol (0.2%) diet together with an LXR agonist (T0901317), ABCG5 and ABCG8 expression was increased in the liver and small intestine [63]. Since the effect was diminished in LXRα-, and LXRα/LXRβ knockout mice, LXR is involved in upregulating the expression of ABCG5 and ABCG8 [63]. However, no LXR binding sites have been found in the promoter region of ABCG5 and ABCG8 genes, but potential LXR binding sites have been suggested to be present in the intronic regions of ABCG5 and ABCG8 [62]. The induction by an RXR agonist, LG268, in the small intestine is LXR-dependent, whereas that in the liver includes an LXR-independent mechanism since LG268 could induce the ABCG5/ABCG8 even in the liver of LXR knockout mice [63]. Among the ligands of nuclear receptors forming heterodimer with RXR, chenodeoxycholate, an agonist of farnesoid-X-activated receptor (FXR/NR1H4), could induce the expression of ABCG5 and ABCG8 in the liver [63]. The mechanism underlying induction of ABCG5 and ABCG8 has been suggested to be an indirect one since a synthetic FXR agonist, GW4064, had insignificant effect and the induction occurred through an indirect mechanism involving upregulation of other factors [63].

Sitosterolemia

ABCG5 and ABCG8 have been considered to be functional transporters for the efflux of shellfish- and phytosterols as well as cholesterol. This is primarily based on the clinical symptoms of sitosterolemia and in vivo studies using Abcg5/Abcg8 double knockout mice, and in vitro transport study has not been performed yet to elucidate the transport characteristics of ABCG5 and ABCG8.

Homozygous mutations of ABCG5 or ABCG8 have been shown to be associated with sitosterolemia [9, 43, 48], a rare autosomal recessive disorder characterized by hyperabsorption of phytosterol (sitosterol, stigmasterol, and campesterol), shellfish sterols (dehydrocholesterol, C-26 sterol, brassicasterol, and 24-methylene cholesterol), and cholesterol from the intestine, leading to the development of xanthomas. Double knockout of Abcg5 and Abcg8 does not produce any obvious physical abnormalities; however, the hepatic and plasma concentrations of phytosterols, such as sitosterol and campesterol, are significantly increased as expected from the symptoms of sitosterolemia [85]. The cholesterol concentration in the plasma and liver was rather lower in the knockout animals following a low-cholesterol diet (0.02% cholesterol); however the hepatic cholesterol level was dramatically increased when the knockout mice were fed a diet containing 2% cholesterol [85]. Knockout of Abcg5 and Abcg8 increased the absorption of cholestanol, campesterol, and sitosterol, but did not affect that of cholesterol, the biliary excretion of which was markedly reduced in mice fed with a diet containing 0.2 and 2% cholesterol [85]. Considering that ABCG5 and ABCG8 form an obligatory heterodimer, it is reasonable that knockout of Abcg5 or Abcg8 is sufficient to increase the plasma concentration of phytosterols, such as beta-sitosterol and campesterol [34, 60]. In contrast to the in vitro observations [22], Abcg5 protein remained in the apical membrane of the liver and intestine even in Abcg8 knockout mice [34].

References

1. Adachi Y, Suzuki H, Schinkel AH, Sugiyama Y (2005) Role of breast cancer resistance protein (Bcrp1/Abcg2) in the extrusion of glucuronide and sulfate conjugates from enterocytes to intestinal lumen. Mol Pharmacol 67:923–928
2. Akiyama TE, Sakai S, Lambert G, Nicol CJ, Matsusue K, Pimprale S, Lee YH, Ricote M, Glass CK, Brewer HB Jr, Gonzalez FJ (2002) Conditional disruption of the peroxisome proliferator-activated receptor gamma gene in mice results in lowered expression of ABCA1, ABCG1, and apoE in macrophages and reduced cholesterol efflux. Mol Cell Biol 22:2607–2619
3. Allen JD, Schinkel AH (2002) Multidrug resistance and pharmacological protection mediated by the breast cancer resistance protein (BCRP/ABCG2). Mol Cancer Ther 1:427–434
4. Allen JD, Brinkhuis RF, Wijnholds J, Schinkel AH (1999) The mouse Bcrp1/Mxr/Abcp gene: amplification and overexpression in cell lines selected for resistance to topotecan, mitoxantrone, or doxorubicin. Cancer Res 59:4237–4241
5. Allen JD, van Loevezijn A, Lakhai JM, van der Valk M, van Tellingen O, Reid G, Schellens JH, Koomen GJ, Schinkel AH (2002) Potent and specific inhibition of the breast cancer resistance protein multidrug transporter in vitro and in mouse intestine by a novel analogue of fumitremorgin C. Mol Cancer Ther 1:417–425

6. Allikmets R, Schriml LM, Hutchinson A, Romano-Spica V, Dean M (1998) A human placenta-specific ATP-binding cassette gene (ABCP) on chromosome 4q22 that is involved in multidrug resistance. Cancer Res 58:5337–5339
7. Annilo T, Tammur J, Hutchinson A, Rzhetsky A, Dean M, Allikmets R (2001) Human and mouse orthologs of a new ATP-binding cassette gene, ABCG4. Cytogenet Cell Genet 94:196–201
8. Bailey-Dell KJ, Hassel B, Doyle LA, Ross DD (2001) Promoter characterization and genomic organization of the human breast cancer resistance protein (ATP-binding cassette transporter G2) gene. Biochim Biophys Acta 1520:234–241
9. Berge KE, Tian H, Graf GA, Yu L, Grishin NV, Schultz J, Kwiterovich P, Shan B, Barnes R, Hobbs HH (2000) Accumulation of dietary cholesterol in sitosterolemia caused by mutations in adjacent ABC transporters. Science 290:1771–1775
10. Breedveld P, Zelcer N, Pluim D, Sonmezer O, Tibben MM, Beijnen JH, Schinkel AH, van Tellingen O, Borst P, Schellens JH (2004) Mechanism of the pharmacokinetic interaction between methotrexate and benzimidazoles: potential role for breast cancer resistance protein in clinical drug–drug interactions. Cancer Res 64:5804–5811
11. Breedveld P, Pluim D, Cipriani G, Wielinga P, van Tellingen O, Schinkel AH, Schellens JH (2005) The effect of Bcrp1 (Abcg2) on the in vivo pharmacokinetics and brain penetration of imatinib mesylate (Gleevec): implications for the use of breast cancer resistance protein and P-glycoprotein inhibitors to enable the brain penetration of imatinib in patients. Cancer Res 65:2577–2582
12. Burris TP, Eacho PI, Cao G (2002) Genetic disorders associated with ATP binding cassette cholesterol transporters. Mol Genet Metab 77:13–20
13. Chen H, Rossier C, Lalioti MD, Lynn A, Chakravarti A, Perrin G, Antonarakis SE (1996) Cloning of the cDNA for a human homologue of the Drosophila white gene and mapping to chromosome 21q22.3. Am J Hum Genet 59:66–75
14. Croop JM, Tiller GE, Fletcher JA, Lux ML, Raab E, Goldenson D, Son D, Arciniegas S, Wu RL (1997) Isolation and characterization of a mammalian homolog of the Drosophila white gene. Gene 185:77–185
15. de Jong FA, Marsh S, Mathijssen RH, King C, Verweij J, Sparreboom A, McLeod HL (2004) ABCG2 pharmacogenetics: ethnic differences in allele frequency and assessment of influence on irinotecan disposition. Clin Cancer Res 10:5889–5894
16. Doyle LA, Ross DD (2003) Multidrug resistance mediated by the breast cancer resistance protein BCRP (ABCG2). Oncogene 22:7340–7358
17. Doyle LA, Yang W, Abruzzo LV, Krogmann T, Gao Y, Rishi AK, Ross DD (1998) A multidrug resistance transporter from human MCF-7 breast cancer cells. Proc Natl Acad Sci USA 95:15665–15670
18. Dreesen TD, Johnson DH, Henikoff S (1988) The brown protein of Drosophila melanogaster is similar to the white protein and to components of active transport complexes. Mol Cell Biol 8:5206–5215
19. Ebert B, Seidel A, Lampen A (2005) Identification of BCRP as transporter of benzo[a]pyrene conjugates metabolically formed in Caco-2 cells and its induction by Ah-receptor agonists. Carcinogenesis 26:1754–1763
20. Ee PL, Kamalakaran S, Tonetti D, He X, Ross DD, Beck WT (2004) Identification of a novel estrogen response element in the breast cancer resistance protein (ABCG2) gene. Cancer Res 64:1247–1251
21. Engel T, Lorkowski S, Lueken A, Rust S, Schluter B, Berger G, Cullen P, Assmann G (2001) The human ABCG4 gene is regulated by oxysterols and retinoids in monocyte-derived macrophages. Biochem Biophys Res Commun 288:483–488
22. Graf GA, Li WP, Gerard RD, Gelissen I, White A, Cohen JC, Hobbs HH (2002) Coexpression of ATP-binding cassette proteins ABCG5 and ABCG8 permits their transport to the apical surface. J Clin Invest 110:659–669
23. Hazard SE, Patel SB (2006) Sterolins ABCG5 and ABCG8: regulators of whole body dietary sterols. Pflugers Arch 1–8
24. Henriksen U, Fog JU, Litman T, Gether U (2005) Identification of intra- and intermolecular disulfide bridges in the multidrug resistance transporter ABCG2. J Biol Chem 280:36926–36934
25. Hirano M, Maeda K, Matsushima S, Nozaki Y, Kusuhara H, Sugiyama Y (2005) Involvement of BCRP (ABCG2) in the biliary excretion of pitavastatin. Mol Pharmacol 68:800–807
26. Honjo Y, Hrycyna CA, Yan QW, Medina-Perez WY, Robey RW, van de Laar A, Litman T, Dean M, Bates SE (2001) Acquired mutations in the MXR/BCRP/ABCP gene alter substrate specificity in MXR/BCRP/ABCP-overexpressing cells. Cancer Res 61:6635–6639
27. Imai Y, Nakane M, Kage K, Tsukahara S, Ishikawa E, Tsuruo T, Miki Y, Sugimoto Y (2002) C421A polymorphism in the human breast cancer resistance protein gene is associated with low expression of Q141K protein and low-level drug resistance. Mol Cancer Ther 1:611–616
28. Imai Y, Ishikawa E, Asada S, Sugimoto Y (2005) Estrogen-mediated post transcriptional down-regulation of breast cancer resistance protein/ABCG2. Cancer Res 65:596–604
29. Jonker JW, Buitelaar M, Wagenaar E, Van Der Valk MA, Scheffer GL, Scheper RJ, Plosch T, Kuipers F, Elferink RP, Rosing H, Beijnen JH, Schinkel AH (2002) The breast cancer resistance protein protects against a major chlorophyll-derived dietary phototoxin and protoporphyria. Proc Natl Acad Sci USA 99:15649–15654
30. Jonker JW, Merino G, Musters S, van Herwaarden AE, Bolscher E, Wagenaar E, Mesman E, Dale TC, Schinkel AH (2005) The breast cancer resistance protein BCRP (ABCG2) concentrates drugs and carcinogenic xenotoxins into milk. Nat Med 11:127–129
31. Kage K, Tsukahara S, Sugiyama T, Asada S, Ishikawa E, Tsuruo T, Sugimoto Y (2002) Dominant-negative inhibition of breast cancer resistance protein as drug efflux pump through the inhibition of S–S dependent homodimerization. Int J Cancer 97:626–630
32. Kennedy MA, Venkateswaran A, Tarr PT, Xenarios I, Kudoh J, Shimizu N, Edwards PA (2001) Characterization of the human ABCG1 gene: liver X receptor activates an internal promoter that produces a novel transcript encoding an alternative form of the protein. J Biol Chem 276:39438–39447
33. Kennedy MA, Barrera GC, Nakamura K, Baldan A, Tarr P, Fishbein MC, Frank J, Francone OL, Edwards PA (2005) ABCG1 has a critical role in mediating cholesterol efflux to HDL and preventing cellular lipid accumulation. Cell Metab 1:121–131
34. Klett EL, Lu K, Kosters A, Vink E, Lee MH, Altenburg M, Shefer S, Batta AK, Yu H, Chen J, Klein R, Looije N, Oude-Elferink R, Groen AK, Maeda N, Salen G, Patel SB (2004) A mouse model of sitosterolemia: absence of Abcg8/sterolin-2 results in failure to secrete biliary cholesterol. BMC Med 2:5
35. Klucken J, Buchler C, Orso E, Kaminski WE, Porsch-Ozcurumez M, Liebisch G, Kapinsky M, Diederich W, Drobnik W, Dean M, Allikmets R, Schmitz G (2000) ABCG1 (ABC8), the human homolog of the Drosophila white gene, is a regulator of macrophage cholesterol and phospholipid transport. Proc Natl Acad Sci USA 97:817–822
36. Kobayashi D, Ieiri I, Hirota T, Takane H, Maegawa S, Kigawa J, Suzuki H, Nanba E, Oshimura M, Terakawa N, Otsubo K, Mine K, Sugiyama Y (2005) Functional assessment of ABCG2 (BCRP) gene polymorphisms to protein expression in human placenta. Drug Metab Dispos 33:94–101

37. Kondo C, Suzuki H, Itoda M, Ozawa S, Sawada J, Kobayashi D, Ieiri I, Mine K, Ohtsubo K, Sugiyama Y (2004) Functional analysis of SNPs variants of BCRP/ABCG2. Pharm Res 21:1895–1903
38. Kondo C, Onuki R, Kusuhara H, Suzuki H, Suzuki M, Okudaira N, Kojima M, Ishiwata K, Jonker JW, Sugiyama Y (2005) Lack of improvement of oral absorption of ME3277 by prodrug formation is ascribed to the intestinal efflux mediated by breast cancer resistant protein (BCRP/ABCG2). Pharm Res 22:613–618
39. Krishnamurthy P, Schuetz JD (2006) Role of ABCG2/BCRP in biology and medicine. Annu Rev Pharmacol Toxicol 46:381–410
40. Krishnamurthy P, Ross DD, Nakanishi T, Bailey-Dell K, Zhou S, Mercer KE, Sarkadi B, Sorrentino BP, Schuetz JD (2004) The stem cell marker Bcrp/ABCG2 enhances hypoxic cell survival through interactions with heme. J Biol Chem 279:24218–24225
41. Laffitte BA, Repa JJ, Joseph SB, Wilpitz DC, Kast HR, Mangelsdorf DJ, Tontonoz P (2001) LXRs control lipid-inducible expression of the apolipoprotein E gene in macrophages and adipocytes. Proc Natl Acad Sci USA 98:507–512
42. Langmann T, Porsch-Ozcurumez M, Unkelbach U, Klucken J, Schmitz G (2000) Genomic organization and characterization of the promoter of the human ATP-binding cassette transporter-G1 (ABCG1) gene. Biochim Biophys Acta 1494:175–180
43. Lee MH, Lu K, Hazard S, Yu H, Shulenin S, Hidaka H, Kojima H, Allikmets R, Sakuma N, Pegoraro R, Srivastava AK, Salen G, Dean M, Patel SB (2001) Identification of a gene, ABCG5, important in the regulation of dietary cholesterol absorption. Nat Genet 27:79–83
44. Lee YJ, Kusuhara H, Jonker JW, Schinkel AH, Sugiyama Y (2005) Investigation of efflux transport of dehydroepiandrosterone sulfate and mitoxantrone at the mouse blood–brain barrier: a minor role of breast cancer resistance protein. J Pharmacol Exp Ther 312:44–52
45. Li AC, Binder CJ, Gutierrez A, Brown KK, Plotkin CR, Pattison JW, Valledor AF, Davis RA, Willson TM, Witztum JL, Palinski W, Glass CK (2004) Differential inhibition of macrophage foam-cell formation and atherosclerosis in mice by PPARalpha, beta/delta, and gamma. J Clin Invest 114:1564–1576
46. Litman T, Brangi M, Hudson E, Fetsch P, Abati A, Ross DD, Miyake K, Resau JH, Bates SE (2000) The multidrug-resistant phenotype associated with overexpression of the new ABC half-transporter, MXR (ABCG2). J Cell Sci 113(Pt 11):2011–2021
47. Lorkowski S, Rust S, Engel T, Jung E, Tegelkamp K, Galinski EA, Assmann G, Cullen P (2001) Genomic sequence and structure of the human ABCG1 (ABC8) gene. Biochem Biophys Res Commun 280:121–131
48. Lu K, Lee MH, Hazard S, Brooks-Wilson A, Hidaka H, Kojima H, Ose L, Stalenhoef AF, Mietinnen T, Bjorkhem I, Bruckert E, Pandya A, Brewer HB Jr, Salen G, Dean M, Srivastava A, Patel SB (2001) Two genes that map to the STSL locus cause sitosterolemia: genomic structure and spectrum of mutations involving sterolin-1 and sterolin-2, encoded by ABCG5 and ABCG8, respectively. Am J Hum Genet 69:278–290
49. Maliepaard M, van Gastelen MA, de Jong LA, Pluim D, van Waardenburg RC, Ruevekamp-Helmers MC, Floot BG, Schellens JH (1999) Overexpression of the BCRP/MXR/ABCP gene in a topotecan-selected ovarian tumor cell line. Cancer Res 59:4559–4563
50. Maliepaard M, Scheffer GL, Faneyte IF, van Gastelen MA, Pijnenborg AC, Schinkel AH, van De Vijver MJ, Scheper RJ, Schellens JH (2001) Subcellular localization and distribution of the breast cancer resistance protein transporter in normal human tissues. Cancer Res 61:3458–3464
51. Merino G, Jonker JW, Wagenaar E, van Herwaarden AE, Schinkel AH (2005) The breast cancer resistance protein (BCRP/ABCG2) affects pharmacokinetics, hepatobiliary excretion, and milk secretion of the antibiotic nitrofurantoin. Mol Pharmacol 67:1758–1764
52. Merino G, Alvarez AI, Pulido MM, Molina AJ, Schinkel AH, Prieto JG (2006) Breast cancer resistance protein (BCRP/ABCG2) transports fluoroquinolone antibiotics and affects their oral availability, pharmacokinetics, and milk secretion. Drug Metab Dispos 34:690–695
53. Mickley L, Jain P, Miyake K, Schriml LM, Rao K, Fojo T, Bates S, Dean M (2001) An ATP-binding cassette gene (ABCG3) closely related to the multidrug transporter ABCG2 (MXR/ABCP) has an unusual ATP-binding domain. Mamm Genome 12:86–88
54. Mitomo H, Kato R, Ito A, Kasamatsu S, Ikegami Y, Kii I, Kudo A, Kobatake E, Sumino Y, Ishikawa T (2003) A functional study on polymorphism of the ATP-binding cassette transporter ABCG2: critical role of arginine-482 in methotrexate transport. Biochem J 373:767–774
55. Mizuarai S, Aozasa N, Kotani H (2004) Single nucleotide polymorphisms result in impaired membrane localization and reduced atpase activity in multidrug transporter ABCG2. Int J Cancer 109:238–246
56. Mizuno N, Suzuki M, Kusuhara H, Suzuki H, Takeuchi K, Niwa T, Jonker JW, Sugiyama Y (2004) Impaired renal excretion of 6-hydroxy-5,7-dimethyl-2-methylamino-4-(3-pyridylmethyl) benzothiazole (E3040) sulfate in breast cancer resistance protein (BCRP1/ABCG2) knockout mice. Drug Metab Dispos 32:898–901
57. Moffit JS, Aleksunes LM, Maher JM, Scheffer GL, Klaassen CD, Manautou JE (2006) 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 317:537–545
58. Nakamura K, Kennedy MA, Baldan A, Bojanic DD, Lyons K, Edwards PA (2004) Expression and regulation of multiple murine ATP-binding cassette transporter G1 mRNAs/isoforms that stimulate cellular cholesterol efflux to high density lipoprotein. J Biol Chem 279:45980–45989
59. Ozvegy-Laczka C, Hegedus T, Varady G, Ujhelly O, Schuetz JD, Varadi A, Keri G, Orfi L, Nemet K, Sarkadi B (2004) High-affinity interaction of tyrosine kinase inhibitors with the ABCG2 multidrug transporter. Mol Pharmacol 65:1485–1495
60. Plosch T, Bloks VW, Terasawa Y, Berdy S, Siegler K, Van Der Sluijs F, Kema IP, Groen AK, Shan B, Kuipers F, Schwarz M (2004) Sitosterolemia in ABC-transporter G5-deficient mice is aggravated on activation of the liver-X receptor. Gastroenterology 126:290–300
61. Rabindran SK, Ross DD, Doyle LA, Yang W, Greenberger LM (2000) Fumitremorgin C reverses multidrug resistance in cells transfected with the breast cancer resistance protein. Cancer Res 60:47–50
62. Remaley AT, Bark S, Walts AD, Freeman L, Shulenin S, Annilo T, Elgin E, Rhodes HE, Joyce C, Dean M, Santamarina-Fojo S, Brewer HB Jr (2002) Comparative genome analysis of potential regulatory elements in the ABCG5-ABCG8 gene cluster. Biochem Biophys Res Commun 295:276–282
63. Repa JJ, Berge KE, Pomajzl C, Richardson JA, Hobbs H, Mangelsdorf DJ (2002) Regulation of ATP-binding cassette sterol transporters ABCG5 and ABCG8 by the liver X receptors alpha and beta. J Biol Chem 277:18793–18800
64. Robey RW, Medina-Perez WY, Nishiyama K, Lahusen T, Miyake K, Litman T, Senderowicz AM, Ross DD, Bates SE (2001) Overexpression of the ATP-binding cassette half-transporter, ABCG2 (Mxr/BCrp/ABCP1), in flavopiridol-resistant human breast cancer cells. Clin Cancer Res 7:145–152
65. Savary S, Denizot F, Luciani M, Mattei M, Chimini G (1996) Molecular cloning of a mammalian ABC transporter homologous to Drosophila white gene. Mamm Genome 7:673–676

66. Scharenberg CW, Harkey MA, Torok-Storb B (2002) The ABCG2 transporter is an efficient Hoechst 33342 efflux pump and is preferentially expressed by immature human hematopoietic progenitors. Blood 99:507–512
67. Schmitz G, Langmann T, Heimerl S (2001) Role of ABCG1 and other ABCG family members in lipid metabolism. J Lipid Res 42:1513–1520
68. Shafran A, Ifergan I, Bram E, Jansen G, Kathmann I, Peters GJ, Robey RW, Bates SE, Assaraf YG (2005) ABCG2 harboring the Gly482 mutation confers high-level resistance to various hydrophilic antifolates. Cancer Res 65:8414–8422
69. Shiozawa K, Oka M, Soda H, Yoshikawa M, Ikegami Y, Tsurutani J, Nakatomi K, Nakamura Y, Doi S, Kitazaki T, Mizuta Y, Murase K, Yoshida H, Ross DD, Kohno S (2004) Reversal of breast cancer resistance protein (BCRP/ABCG2)-mediated drug resistance by novobiocin, a coumermycin antibiotic. Int J Cancer 108:146–151
70. Sparreboom A, Gelderblom H, Marsh S, Ahluwalia R, Obach R, Principe P, Twelves C, Verweij J, McLeod HL (2004) Diflomotecan pharmacokinetics in relation to ABCG2 421C>A genotype. Clin Pharmacol Ther 76:38–44
71. Sullivan DT, Bell LA, Paton DR, Sullivan MC (1979) Purine transport by malpighian tubules of pteridine-deficient eye color mutants of Drosophila melanogaster. Biochem Genet 17:565–573
72. Suzuki M, Suzuki H, Sugimoto Y, Sugiyama Y (2003) ABCG2 transports sulfated conjugates of steroids and xenobiotics. J Biol Chem 278:22644–22649
73. Tearle RG, Belote JM, McKeown M, Baker BS, Howells AJ (1989) Cloning and characterization of the scarlet gene of Drosophila melanogaster. Genetics 122:595–606
74. van Herwaarden AE, Schinkel AH (2006) The function of breast cancer resistance protein in epithelial barriers, stem cells and milk secretion of drugs and xenotoxins. Trends Pharmacol Sci 27:10–16
75. van Herwaarden AE, Jonker JW, Wagenaar E, Brinkhuis RF, Schellens JH, Beijnen JH, Schinkel AH (2003) The breast cancer resistance protein (Bcrp1/Abcg2) restricts exposure to the dietary carcinogen 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine. Cancer Res 63:6447–6452
76. van Herwaarden AE, Wagenaar E, Karnekamp B, Merino G, Jonker JW, Schinkel AH (2006) Breast cancer resistance protein (Bcrp1/Abcg2) reduces systemic exposure of the dietary carcinogens aflatoxin B1, IQ and Trp-P-1 but also mediates their secretion into breast milk. Carcinogenesis 27:123–130
77. Vaughan AM, Oram JF (2005) ABCG1 redistributes cell cholesterol to domains removable by high density lipoprotein but not by lipid-depleted apolipoproteins. J Biol Chem 280:30150–30157
78. Volk EL, Schneider E (2003) Wild-type breast cancer resistance protein (BCRP/ABCG2) is a methotrexate polyglutamate transporter. Cancer Res 63:5538–5543
79. Volk EL, Rohde K, Rhee M, McGuire JJ, Doyle LA, Ross DD, Schneider E (2000) Methotrexate cross-resistance in a mitoxantrone-selected multidrug-resistant MCF7 breast cancer cell line is attributable to enhanced energy-dependent drug efflux. Cancer Res 60:3514–3521
80. Volk EL, Farley KM, Wu Y, Li F, Robey RW, Schneider E (2002) Overexpression of wild-type breast cancer resistance protein mediates methotrexate resistance. Cancer Res 62:5035–5040
81. Wang H, Zhou L, Gupta A, Vethanayagam RR, Zhang Y, Unadkat JD, Mao Q (2006) Regulation of BCRP/ABCG2 expression by progesterone and 17beta-estradiol in human placental BeWo cells. Am J Physiol Endocrinol Metab 290:E798–E807
82. Wang N, Lan D, Chen W, Matsuura F, Tall AR (2004) ATP-binding cassette transporters G1 and G4 mediate cellular cholesterol efflux to high-density lipoproteins. Proc Natl Acad Sci USA 101:9774–9779
83. Wittenburg H, Carey MC (2002) Biliary cholesterol secretion by the twinned sterol half-transporters ABCG5 and ABCG8. J Clin Invest 110:605–609
84. Xu J, Liu Y, Yang Y, Bates S, Zhang JT (2004) Characterization of oligomeric human half-ABC transporter ATP-binding cassette G2. J Biol Chem 279:19781–19789
85. Yu L, Hammer RE, Li-Hawkins J, Von Bergmann K, Lutjohann D, Cohen JC, Hobbs HH (2002) Disruption of Abcg5 and Abcg8 in mice reveals their crucial role in biliary cholesterol secretion. Proc Natl Acad Sci USA 99:16237–16242
86. Zaher H, Khan AA, Palandra J, Brayman TG, Yu L, Ware JA (2006) Breast cancer resistance protein (Bcrp/abcg2) is a major determinant of sulfasalazine absorption and elimination in the mouse. Mol Pharmacol 3:55–61
87. Zamber CP, Lamba JK, Yasuda K, Farnum J, Thummel K, Schuetz JD, Schuetz EG (2003) Natural allelic variants of breast cancer resistance protein (BCRP) and their relationship to BCRP expression in human intestine. Pharmacogenetics 13:19–28
88. Zhang W, Mojsilovic-Petrovic J, Andrade MF, Zhang H, Ball M, Stanimirovic DB (2003) The expression and functional characterization of ABCG2 in brain endothelial cells and vessels. FASEB J 17:2085–2087
89. Zhang W, Yu BN, He YJ, Fan L, Li Q, Liu ZQ, Wang A, Liu YL, Tan ZR, Fen J, Huang YF, Zhou HH (2006) Role of BCRP 421C>A polymorphism on rosuvastatin pharmacokinetics in healthy Chinese males. Clin Chim Acta. DOI 10.1016/j.aca.2006.05.010
90. Zhou S, Schuetz JD, Bunting KD, Colapietro AM, Sampath J, Morris JJ, Lagutina I, Grosveld GC, Osawa M, Nakauchi H, Sorrentino BP (2001) The ABC transporter Bcrp1/ABCG2 is expressed in a wide variety of stem cells and is a molecular determinant of the side-population phenotype. Nat Med 7: 1028–1034
91. Zhou S, Morris JJ, Barnes Y, Lan L, Schuetz JD, Sorrentino BP (2002) Bcrp1 gene expression is required for normal numbers of side population stem cells in mice, and confers relative protection to mitoxantrone in hematopoietic cells in vivo. Proc Natl Acad Sci USA 99:12339–12344