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REVIEW

The ABCG family of membrane-associated transporters: you don't have to be big to be mighty

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Along with many other mammalian ATP-binding cassette (ABC) transporters, members of the ABCG group are involved in the regulated transport of hydrophobic compounds across cellular membranes. In humans, five ABCG family members have been identified, encoding proteins ranging from 638 to 678 amino acids in length. All five have been the subject of intensive investigation to better understand their physiological roles, expression patterns, interactions with substrates and inhibitors, and regulation at both the transcript and protein level. The principal substrates for at least four of the ABCG proteins are endogenous and dietary lipids, with ABCG1 implicated in particular in the export of cholesterol, and ABCG5 and G8 forming a functional heterodimer responsible for plant sterol elimination from the body. ABCG2 has a much broader substrate specificity and its ability to transport numerous diverse pharmaceuticals has implications for the absorption, distribution, metabolism, excretion and toxicity (ADMETOx) profile of these compounds. ABCG2 is one of at least three so-called multidrug resistant ABC transporters expressed in humans, and its activity is associated with decreased efficacy of anti-cancer agents in several carcinomas. In addition to its role in cancer, ABCG2 also plays a role in the normal physiological transport of urate and haem, the implications of which are described. We summarize here data on all five human ABCG transporters and provide a current perspective on their roles in human health and disease.

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Abbreviations
ABC, ATP-binding cassette; ADEMTOx, absorption distribution metabolism excretion and toxicity; AML, acute myeloid leukaemia; CMC, critical micelle concentration; CYP, cytochrome P450; HDL, high-density lipoproteins; LXR, liver X-receptor; MDR, multidrug resistance; miRNA, microRNA; NBD, nucleotide binding domain; SNP, single nucleotide polymorphism

Introduction

There are almost 50 ATP-binding cassette (ABC) proteins encoded in the human genome, making them one of the largest families of membrane proteins. Comparative sequence analysis of the genomes of multiple eukaryotes has enabled us to assign ABC sequences to one of seven principal different subfamilies, ABCA-ABCG. Of these, two are not membrane associated and are not involved in transport (ABCE and ABCF), instead being implicated in translational control. The remaining five subfamilies all contain several membrane proteins involved in cellular transport processes (Figure 1). The functions of ABC transporters of the ABCA, ABCB, ABCC and ABCD subfamilies are covered in other reviews in this issue, and are not discussed at any length here. The focus of our review is the five member subfamily ABCG. Although the exact substrate specificities for some of the five proteins remain ill defined it is clear that all five ABCG proteins are implicated in the transport of lipids. In this respect the ABCG family would seem to have a narrower functionality than the ABCB and ABCC families, which encode peptide and cyclic nucleotide transporters, chloride ion channels and channel regulators, as well as transporters of hydrophobic substrates (lipids, bile salts, drugs). However, at least one ABCG family

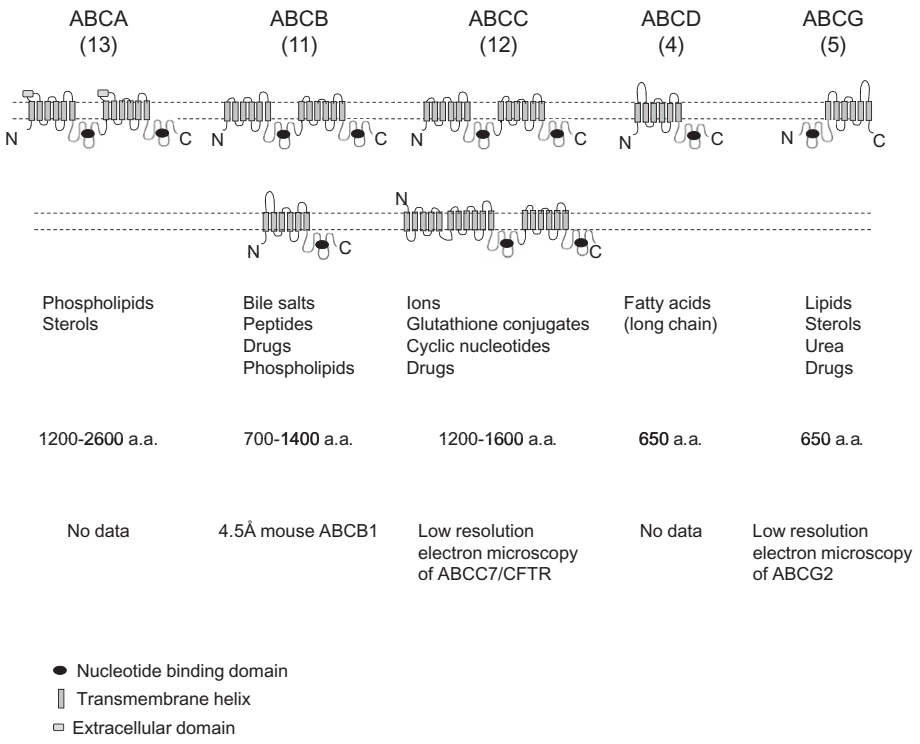

Figure 1

Architecture of human ABC proteins. Each of the five subfamilies of human ABC proteins involved in transmembrane transport is listed at the top of a panel with the number of members identified in the human genome in parenthesis. The transmembrane architecture is then shown in schematic fashion, with the N- and C-terminus of the primary sequence indicated. The approximate position of the membrane is denoted by a dotted line. Where members of a subfamily show different architectures (e.g. ABCB and ABCC), two alternatives are shown for illustrative purposes. Several ABCA proteins are distinct in having large extracellular domains not seen in any other ABC protein sequences (and with no homology to other known sequences).Underneath each topology diagram the spectrum of substrates (where characterized) is indicated, followed by the size range of the proteins (in amino acids; a.a.), followed by the highest resolution structural data obtained to date. For more information refer to other review articles in this series. The two non-transport associated families ABCE (1 member) and ABCF (2 members) are not shown here for clarity.

member (ABCG2) displays a broad substrate specificity, consistent with it being a multidrug efflux pump. Here we attempt to bring together the pharmacology of these five transporters with data on their biochemistry and their physiological role. In particular, we try to highlight the functional dependence of ABCG proteins with other membrane proteins, and illustrate that their regulation may be shared with many other proteins involved in cellular lipid homeostasis.

Pharmacology and identification of ABCG transporter substrates

A key aim in understanding the function of ABCG transporters (Table 1) is the identification of substrates and measurement of quantitative interaction with the protein. Presently, this is hampered by several considerations, not the least of which being the difficulty in characterizing the pharmacology of lipid transporters. Additionally, there is a degree of substrate overlap and substrate promiscuity in the ABCG transporters making it difficult to identify selective pharmacological agents. This broad substrate specificity in ABCG2 is a feature shared with two other human ABC proteins (ABCB1 and ABCC1), and all three are known as multidrug pumps reflecting this.

ABCG1 has been shown to be involved in the export of a variety of lipids (in conjunction with ABCA1 – see below), with most emphasis being placed on cholesterol due to its role in the development of atherosclerosis. Quantitative analysis remains elusive, but suggested lipid export products of ABCG1 include the cholesterol precursor desmosterol, cholesterol oxidation products (7-keto cholesterol, 7-β hydroxy cholesterol and the side chain oxidized sterols 24-hydroxy, 25-hydroxy and 27-hydroxy cholesterol) as well as the choline-containing phospholipids sphingomyelin and phosphatidylcholine (Kobayashi *et al.*, 2006; Engel *et al.*, 2007; Terasaka *et al.*, 2007; Wang *et al.*, 2008; Tarling *et al.*, 2010). Only one study has so far investigated the direct interaction of ABCG1 with a large number of potential substrates by expressing the protein in a recombinant system (baculovirus-infected Sf9 cells) and then analysing the ability of putative substrates to stimulate the ATPase activity of ABCG1-containing membrane fractions (Cserepes *et al.*, 2004). Interestingly, sterols did not stimulate ATPase activity and the only substrate identified using this system was the dye Rhodamine123. However, a number of potential ABCG1 inhibitors were identified including cyclosporine A, benzamil

Table 1
The human ABCG transporters

Name	Chromosome location	Primary sequence[a]	Dimerization	Functional interaction with other ABCs	Principal substrates
ABCG1	21q22	638–678	Homo; possibly hetero with ABCG4	ABCA1	Lipids
ABCG2	4q21–4q22	655	Homo-	n.d.	Urate, haem, drugs
ABCG4	11q23	646	Homo; possibly hetero with ABCG1	n.d	Lipids
ABCG5	2p21	651	Obligate hetero with G8	ABCB4	Plant sterols
ABCG8	2p21	673	Obligate hetero with G5	ABCB4	Plant sterols

[a]Number of amino acids; n.d., no functional interaction with other ABC proteins has been reported.

and L-thyroxine. The same study failed to identify any substrates or inhibitors for ABCG4; however, the authors suggested that the two ABC transporters could potentially heterodimerize (Cserepes *et al.*, 2004). Overexpression studies have also suggested that ABCG4 activity leads to export of cholesterol as well as the cholesterol precursor desmosterol (Wang *et al.*, 2008).

Studies of ABCG2 *in vitro* and *in vivo* have identified a vast array of substrates and inhibitors (summarized in Table 2), and here we limit ourselves to discussion of the interaction of anti-cancer agents with ABCG2, as determined by quantitative radioligand binding of ABCG2 overexpressed in Hi-5 insect cells (Clark *et al.*, 2006). Although performed with a mutant isoform of ABCG2 (Arg482Gly) this study remains the only direct determination of substrate binding affinities in a human ABCG transporter. The radiolabelled anti-cancer agent daunomycin bound to ABCG2-containing membranes with a dissociation constant of *c.* 100 nM. Co-operativity of binding was observed, consistent with sites for daunomycin binding on each ABCG2 monomer in a dimer (Clark *et al.*, 2006). The bound drug could be displaced in a homologous or heterologous manner by unlabelled anti-cancer drugs (doxorubicin, mitoxantrone) with potencies (IC_{50}) in the low micromolar range. Prazosin displayed much lower potency while methotrexate (which is not transported by the R482G isoform) was unable to displace the bound radioligand (Clark *et al.*, 2006). The data support the hypothesis that, in common with other multidrug pumps, ABCG2 contains multiple pharmacologically distinct binding sites. A follow-up study revealed that a switch from high-affinity binding (potentially the drug association step) to low affinity (potentially the drug release step) was observed upon ATP binding, rather than ATP hydrolysis (McDevitt *et al.*, 2008). Despite the large number of studies identifying substrates and inhibitors of ABCG2 (Table 2) there is still a lack of information regarding which residues might form the substrate binding sites, although in addition to Arg482 mentioned above, there has been a proposed identification of a steroid binding site in ABCG2, based upon homology to oestrogen receptor sequences (Velamakanni *et al.*, 2008).

Studies identifying substrates and inhibitors of ABCG5 and G8 have also utilized protein overexpression systems, including insect Sf9 cells, methylotrophic yeast and lentiviral infection of epithelial cells (Wang *et al.*, 2006a; Vrins *et al.*, 2007; Johnson *et al.*, 2010), followed by reconstitution into proteoliposomes to enable analysis of substrate specificity and activity. Such studies rely on two methods to analyse protein function. The first is the stimulation of ATPase activity by putative substrates, and the second is the transport of substrates measured in a vesicle system. ABCG5/G8 protein purified and reconstituted from a high level Pichia expression system displayed ATPase activity that was stimulated 8- to 40-fold by a range of bile acids with maximal stimulation induced by taurocholate, cholate and glycocholate (Johnson *et al.*, 2010). In contrast, ATPase activity was not stimulated by sterols (Wang *et al.*, 2006a). The half-maximal concentrations required for bile-acid stimulation (EC_{50}) were in the millimolar range (10–20 mM for the bile acids mentioned), which is comparable with their critical micelle concentrations (CMC) and also to physiologically relevant concentrations. So are bile acids a likely substrate of ABCG5/G8, or is the situation more complex than this? Of note then are studies of sterol transport by ABCG5/G8, which have utilized inside-out vesicles produced from epithelial cells transduced with lentiviral vectors (Vrins *et al.*, 2007). Cholesterol transport into the vesicles was not measurable in the absence of bile acids, but their presence at levels comparable to the CMC was enough to stimulate many fold the rate of cholesterol transport. Sub-millimolar concentrations of phosphatidylcholine stimulated further cholesterol transport (Vrins *et al.*, 2007). Taken together a possible model for G5/G8 activity in the hepatobiliary clearance of sterols has been proposed in which the transporter may act to mediate the transfer of sterols into mixed micelles of bile acids and phospholipids (Vrins *et al.*, 2007; Johnson *et al.*, 2010), although the exact underlying mechanism remains unconfirmed.

Tissue distribution and sub-cellular localization

ABCG1 mRNA and protein expression is widespread but highest in macrophage-rich tissue such as spleen, lung, and thymus as well as in brain (Chen *et al.*, 1996; Savary *et al.*, 1996). Functional hetero-dimerization with ABCG4 has been proposed (but not substantiated) and it is of interest that several cell types in the brain, mainly neurons and astrocytes, express ABCG1 as well as ABCG4 mRNA with the highest basal expression of both transporters in neurons (Tarr and

Table 2

Substrates, modulators and inhibitors of ABCG2

Classification	Example compounds	Affinity/potency for effect	Reference
Antibiotics	Ciprofloxacin Norfloxacin		(Merino *et al.*, 2006)
Anti-cancer drugs	Daunomycin Etoposide Doxorubicin Erlotinib, gefitinib Mitoxantrone Topotecan	K_D 100 nM in radioligand binding studies IC_{50} of 0.75 μM to displace daunomycin binding Distinct effects shown for different tyrosine kinase inhibitors at low μM IC_{50} of 1.5 μM to displace daunomycin binding.	(Clark *et al.*, 2006) (Clark *et al.*, 2006) (Hegedus *et al.*, 2009; Dohse *et al.*, 2010) (Clark *et al.*, 2006)
Sterols	Estradiol, cholesterol, progesterone	EC_{50} values for ATPase stimulation of 5–20 μM	(Janvilisri *et al.*, 2003)
Antihypertensive drugs	Telmisartan Prazosin 1,4 dihydropyridines	IC_{50} of 17 μM for inhibition of ABCG2 activity High μM IC_{50} to displace daunomycin binding IC_{50} of 5 μM for nicardipine inhibition of ABCG2 activity.	(Weiss *et al.*, 2010) (Clark *et al.*, 2006) (Zhang *et al.*, 2005) (Shukla *et al.*, 2006)
HIV-1 protease inhibitors	Abacavir Nelfinavir	High μM IC_{50} for inhibition of ABCG2 activity	(Weiss *et al.*, 2007)
Immuno-suppressants	Cyclosporin A Tacrolimus	Low μM IC_{50} for inhibition of ABCG2 activity	(Pawarode *et al.*, 2007)
Flavonoids	Apigenin Biochanin A	IC_{50} of 0.4 to 40 μM for inhibition of ABCG2 activity	(Ahmed-Belkacem *et al.*, 2005)
Acridones	Elacridar	Sub-micromolar (100nM) IC_{50} for inhibition of ABCG2 activity	(Maliepaard *et al.*, 2001b)
Mycotoxins	Fumitremorgin C Ko143 and derivatives	IC_{50} of 0.1 to 5 μM for inhibition of ABCG2 activity	(Rabindran *et al.*, 2000; Allen *et al.*, 2002)

The break in the table is used to separate transported substrates from compounds that 'inhibit the activity' of the protein. This broad definition typically means the inhibition of fluorescent substrate accumulation (e.g. mitoxantrone) in ABCG2-expressing cell lines, or the reversal of ABCG2-mediated resistance to a cytotoxic compound. For brevity this table does not represent all the substrates of ABCG2 and we apologize to authors whose work has been omitted.

Edwards, 2008). ABCG4 mRNA is predominantly expressed in the brain and the eye, leading to speculation that it performs a so far undefined sterol export function exclusively in these tissues. At the sub-cellular level, both ABCG1 and ABCG4 in brain have been shown to reside exclusively in intracellular vesicles (Tarr and Edwards, 2008) while in various other cell types, they are thought to be localized intra-cellularly as well as on the plasma membrane (Kobayashi *et al.*, 2006; Wang *et al.*, 2006b).

ABCG2 expression (mRNA and protein) has been observed in the placenta, small and large intestine (highest in the duodenum, lowest in the rectum), the central nervous system, liver, adrenal gland, prostate, testes, uterus, brain, prostate, stomach, lung and kidney (Maliepaard *et al.*, 2001a; Gutmann *et al.*, 2005). Within the kidney, small and large intestine, ABCG2 is located at the apical membrane of epithelial cells, while in hepatocytes, it has a canalicular membrane localization, all consistent with roles in excretion processes (Maliepaard *et al.*, 2001a; Huls *et al.*, 2008). At the blood–brain barrier, and placental syncytiotrophoblasts, localization is such that transport of potentially noxious substrates across these barriers would be restricted (Maliepaard *et al.*, 2001a; Zhang *et al.*, 2003). For example, ABCG2 is expressed on the luminal side of the brain capillaries, suggesting a brain-to-blood transport direction (Zhang *et al.*, 2003).

Uniquely among the ABCG family, ABCG2 expression is used as a marker for stem cells, its export of the fluorescent compound Hoechst 33342 being responsible for the 'side population' phenotype observed in flow cytometry. Recent studies have shown that ABCG2 is also expressed in tumour initiating stem cells (cancer stem cells). High levels of ABCG2 expression have been detected in cancer stem cells derived from different tumours such as retinoblastoma, lung, liver and pancreatic cancer [reviewed by (Visvader and Lindeman, 2008)].

Unlike ABCG2, which is widely expressed, ABCG5/G8 are exclusively expressed throughout the gastro-intestinal tract intestine, liver and gall bladder. Expression is highest in the small intestine and liver with lower expression levels in the large intestine (Klett *et al.*, 2004; Ismair *et al.*, 2009). The proteins are localized to the apical (i.e. gut-facing) membrane

of enterocytes, consistent with a role in limiting the absorption of dietary sterols (Graf *et al.*, 2003). In the liver and gall bladder, ABCG5 and G8 are found at apical membranes of hepatocyte canaliculi and gall bladder epithelial cells respectively. In the liver it is notable that MRP2 (ABCC2), which is required for effective biliary secretion, is co-localized with ABCG5 and G8 (Klett *et al.*, 2004). Although they do not appear to have completely identical patterns of expression, there is sufficient overlap to support the *in vitro* functional interdependence of the two transporters. At the sub-cellular level there is evidence that the two ABCG5/G8 half-transporters localize to membrane micro-domains [potentially cholesterol-rich domains; (Klett *et al.*, 2004; Ismair *et al.*, 2009)].

Regulation of the ABCG gene expression

Multiple levels of regulation have been documented for ABCG transporters with influences on transcription, mRNA suppression and modulation of protein expression. The transcriptional control of four of the five ABCG genes has been investigated in detail, ABCG4 being the exception, and many transcription factor binding sites have been identified and validated. For several of the ABCG genes, transcriptional regulation occurs through elements responsible for controlling the expression of proteins involved in similar physiological processes. For example, ABCG5/G8 expression is regulated by the nuclear orphan receptor liver receptor homologue-1 (Freeman *et al.*, 2004; Sumi *et al.*, 2007), which also mediates transcription of the bile lipid transporter protein ABCB3 (MDR3), and the bile acid synthesizing enzymes CYP7 and CYP8 (Freeman *et al.*, 2004). Similarly, ABCG1 and ABCG5/8 mRNA expression increases sharply when cells are loaded with cholesterol or when mice are fed on cholesterol-rich diets (Repa *et al.*, 2002). This expression is highly activated by ligands for the liver X receptors (LXR) (Venkateswaran *et al.*, 2000; Repa *et al.*, 2002; Graf *et al.*, 2003; Pawar *et al.*, 2003; Calpe-Berdiel *et al.*, 2008), a family of transcription factors that regulate a number of genes involved in cholesterol homeostasis including ABCA1, apolipoprotein E and sterol regulatory element binding protein 1c. Indeed, several highly conserved LXR response elements have been identified in ABCG1 promoter regions (Lorkowski *et al.*, 2001; Sabol *et al.*, 2005). In contrast, ABCG4 does not seem to be an LXR target, and further information regarding the regulation of this intriguing transporter is urgently needed (Tarr and Edwards, 2008). ABCG1 expression is also up-regulated by peroxisome proliferator-activated receptor-agonists (Li *et al.*, 2004), as indeed may be the case for ABCG2 (Szatmari *et al.*, 2006). However, no evidence in favour of peroxisome proliferator-activated receptor activation has been forthcoming for ABCG5/G8 (Repa *et al.*, 2002). ABCG5/G8 are unique in that they share an intergenic region (see below), which means that regulation of both genes' transcription is most likely mediated by common mechanisms.

ABCG2's different functional roles (compared with the other ABCG family members) are potentially reflected in its transcriptional control. The upstream region contains functional oestrogen (Benderra *et al.*, 2004) and hypoxia (Krishnamurthy *et al.*, 2004) response elements, consistent with its expression in mammary glands and a role in haemopoietic stem cell protection. Various other potential transcriptional control points have been identified in the ABCG2 promoter, including putative Sp1, AP1 and AP2 binding sites (Bailey-Dell *et al.*, 2001). Furthermore, ABCG2 expression has been shown to be up-regulated by the aryl hydrocarbon receptor (Ebert *et al.*, 2005), suppressed by DNA methylation (Turner *et al.*, 2006), and, with specific respect to hepatobiliary cancer, up-regulated by Oct4 (Wang *et al.*, 2010).

Post-transcriptionally, the increased awareness of microRNA (miRNA) mediated control of transcript levels has led to the identification of at least three miRNAs that can influence ABCG transporter expression. MiRNAs miR-519c and miRNA-328 decrease the level of ABCG2 mRNA and thus protein by binding to a putative binding site located within the 3′ untranslated region. Intriguingly, these binding sites are proposed to be absent from some drug selected, ABCG2 overexpressing cell lines (Nakanishi *et al.*, 2006; Pan *et al.*, 2009; To *et al.*, 2009). Similarly, miR-520h has also been reported to target ABCG2 in hematopoietic stem cells during their differentiation into progenitor cells (Liao *et al.*, 2008). For ABCG1, a microRNA termed miR-33 was shown to reduce ABCG1 RNA levels in murine, but not in human cells, uncovering interesting species differences (Rayner *et al.*, 2010).

Protein structure and post-translational control

The genetic loci that encode for the ABCG proteins have been identified, and the transcripts and predicted protein structures are established (Table 1). For ABCG1 a number of potentially different transcriptional start sites exist, which when coupled with alternative splicing events, can give rise to multiple isoforms, the largest of which is thought to be the predominant form in macrophages (Lorkowski *et al.*, 2001). Also of note is that the genes encoding ABCG5 and ABCG8 are transcribed in opposite directions with a small, shared intergenic region of 374 bp. All five ABCG genes share a common ancestral gene, and for ABCG5/G8 the genetic structure is evidence for further gene duplication event (Berge *et al.*, 2000; Lee *et al.*, 2001).

The organization of ABCG proteins is atypical compared with other eukaryotic ABC transporters (Figure 1). They contain a single, cytoplasmic, nucleotide binding domain (NBD) at the N-terminus, which is responsible for ATP binding and hydrolysis, and a single transmembrane domain towards the C-terminus, which contains six transmembrane α-helices required for substrate recognition and transport. This basic architecture not only represents just half the required number of domains for a fully functional ABC transporter (hence the ABCG proteins here are often referred to as 'half-transporters'), but also the sequential order of the domains is the opposite to the majority of other ABC transporters, where the NBDs are C-terminal to the transmembrane domains (Figure 1). The significance of this altered topology is currently not understood, and the 3D structure of ABCG proteins remains elusive, with data restricted to

electron microscopy studies of human ABCG2 (McDevitt *et al.*, 2006; Rosenberg *et al.*, 2010). Besides the sequence similarities of the ABC motifs, there is very little homology between the ABCG proteins. ABCG1 and ABCG4 are the exception to this as they are highly homologous in structure (72 % identity at the amino acid level).

The 'half-transporter' topology means that ABCG proteins must partner with another ABCG protein, either by homo-or heterodimerization, in order to function. ABCG1, ABCG2 and ABCG4 can function as homodimers and this is thought to be a physiologically relevant association from *in vitro* single transfection studies (Bhatia *et al.*, 2005; Henriksen *et al.*, 2005; Gelissen *et al.*, 2006; Kobayashi *et al.*, 2006). For ABCG2, however, higher order assemblies have also been proposed (Xu *et al.*, 2004). *In vitro* overexpression studies in insect Sf9 cells have suggested that ABCG1 and ABCG4 can heterodimerize, although any direct evidence for such partnership *in vivo* is still elusive (Cserepes *et al.*, 2004). By contrast, a stream of studies has shown that ABCG5/G8 form obligate heterodimers, with their co-expression being necessary to achieve full glycosylation and stability of the transporters (Graf *et al.*, 2002; 2003; 2004). Structurally, very little is known about the dimerization process, although the NBDs of the two half-transporters are likely to interact in a manner similar to that of other full-sized ABC proteins (Kerr *et al.*, 2010).

Post-translational modifications of ABCG transporters have been identified but the significance of these for functional control remains less clear. ABCG2 and ABCG5/G8 are glycosylated, and for the latter pair, glycosylation is an absolute requirement for proper heterodimerization and protein function (Graf *et al.*, 2002). In the case of ABCG2 a single residue (N596) is glycosylated and this modification is associated with correct assembly and effective trafficking of stable protein to the plasma membrane, but is not required for protein function (Diop and Hrycyna, 2005). Both ABCG1 and ABCG4 are thought to be unglycosylated (Kobayashi *et al.*, 2006; Engel *et al.*, 2007).

Phosphorylation has been implicated in the regulation of ABCG2, with phosphorylation of Thr-362 by Pim-1 kinase affecting the oligomerization and plasma membrane localization of ABCG2, with unknown effects on the substrate specificity of the transporter (Xie *et al.*, 2008). For ABCG1, the only factors so far investigated with regards to control of protein expression via phosphorylation are fatty acid oxidation products generated from the activity of 12/15 lipoxygenase, an enzyme investigated for its involvement in the pathogenesis of atherosclerosis and diabetes. Nagelin *et al.* (Nagelin *et al.*, 2009) showed that the fatty acyl oxidation product 12-HETE increased serine phosphorylation and degradation of ABCG1 protein in murine macrophages. The post-translational control of different ABCG1 isoforms might be distinct as the inclusion of a 12 amino acid peptide due to alternative splicing of the gene reduced the basal half-life of the protein, the mechanisms for which are under investigation (Gelissen *et al.*, 2010).

Protein function

ABCG1 was first described as the human homologue of the Drosophila *white* gene (Chen *et al.*, 1996; Savary *et al.*, 1996), known to be involved in the import of eye pigments precursors in Drosophila. The human ABCG1 gene product, however, was first identified as a lipid transporter, involved in the export of cholesterol and phospholipids from macrophages to high-density lipoproteins [HDL (Klucken *et al.*, 2000)]. It is now becoming clear that the function of ABCG1 extends beyond macrophages to a more general role in cellular lipid homeostasis, including a role in T cells and inflammation, and brain lipid homeostasis. The majority of studies aimed at elucidating the function of ABCG1 have focussed on its role in atherosclerosis, in particular macrophage lipid export. This process forms the first step in the reverse cholesterol transport pathway that exports cholesterol from peripheral cells such as macrophages to circulating lipoproteins for eventual excretion via the liver (Figure 2). Cholesterol export to lipoprotein acceptors is thought to be compromised in macrophage foam cells, one of the early hallmarks of atherosclerosis. ABCG1 is proposed to function in conjunction with ABCA1, a full-sized ABC lipid transporter, whereby ABCA1 initially donates phospholipids and cholesterol to lipid-poor HDL subclasses. These lipid-poor particles are efficient acceptors for ABCG1 mediated lipid export, suggesting that the two transporters can potentially work synergistically (Gelissen *et al.*, 2006; Vaughan and Oram, 2006). Studies in murine models have confirmed that the combined effect of the two transporters is essential for maintaining macrophage lipid homeostasis (Yvan-Charvet *et al.*, 2007; Out *et al.*, 2008). Central to this has been the *Abcg1-/-* mouse, which displays massive accumulation of neutral lipids and phospholipids in hepatocytes and tissue macrophages upon fat feeding (Kennedy *et al.*, 2005), with very pronounced accumulation of lipids in lung macrophages. In terms of atherosclerosis, the *Abcg1-/-* mouse displays a moderate increase in lesion size (approximately twofold) upon challenge with a high fat, high cholesterol diet (Out *et al.*, 2007). The absence of ABCG1 does not affect plasma lipid levels in this model, suggesting that unlike ABCA1, ABCG1 by itself does not contribute to plasma HDL levels (Out *et al.*, 2007). Studies using *Abcg1-/-* mouse models have furthermore implicated a role for ABCG1 in cells other than macrophages. Aortic endothelial cells from *Abcg1-/-* mice, besides displaying reduced cholesterol export to HDL, have increased surface expression of adhesion molecules, leading to an increase in monocyte adhesion (Whetzel *et al.*, 2010). Peripheral lymphocyte and thymocyte proliferation was also observed (Armstrong *et al.*, 2010), while in the brain, several oxysterol species were found to accumulate (Tarling *et al.*, 2010). Whether any of these results translate to a phenotype in humans remains to be seen. Little is so far known about the function of ABCG4 in humans, except that its expression pattern and *in vitro* sterol export capacity suggests a role in lipid homeostasis in brain and possibly in the eye. *Abcg4-/-* mice have very little phenotypic changes; however, when crossed with *Abcg1-/-* mice, brain sterol accumulation becomes prominent and much more pronounced than that seen in the individual knockouts (Wang *et al.*, 2008). This again suggests a potential coordinate role for ABCG1 and ABCG4 in brain.

ABCG2's ability to transport multiple substrates has meant that assigning a single physiological function has proved impossible. It has been proposed to be a major player in restricted intestinal absorption of pharmaceuticals, the

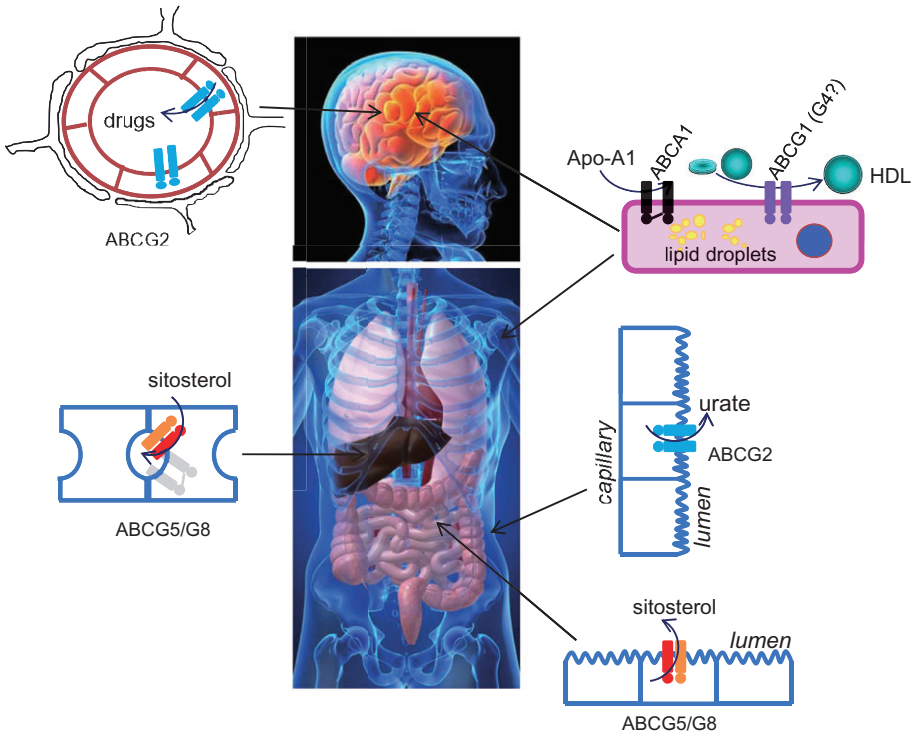

Figure 2

Principal functions of human ABCG transporters. Major functions of several transport systems as described in the text. Clockwise from top left: ABCG2 homodimer (blue) exports drugs into capillary lumen, preventing their crossing the blood–brain barrier. The reverse cholesterol transport pathway in macrophages – ABCA1 (black) activity results in formation of spherical or discoid HDL particles, which are then substrates for cholesterol export by ABCG1 homodimer (purple). A less well understood pathway involving cholesterol transport in the brain is proposed for ABCG4. ABCG2 (blue) in the proximal tubule expels urate into the kidney lumen. ABCG5/G8 (orange/red) in the small intestine prevent plant sterol absorption. ABCG5/G5 (orange/red) combine with ABCB4 (grey) in biliary lipid secretion.

defence of organs (e.g. brain, placenta) against xenobiotics, the protection of haematopoietic stem cells against haem-induced toxicity (Krishnamurthy *et al.*, 2004), the export of nutrients into milk (Vlaming *et al.*, 2009), the export of urate from kidney (Woodward *et al.*, 2009) and the export of chemotherapeutic drugs from cancer cells. Some of these activities (Figure 2) are covered elsewhere in this review, and a more extensive coverage of ABCG2 functions has appeared recently (Vlaming *et al.*, 2009).

ABCG5 or ABCG8 function in the intestinal absorption and biliary elimination of plant sterols as well as cholesterol, defects in which are associated with sitosterolemia (Figure 2 and see Pathology and Clinical Significance). In addition to the *in vitro* studies of protein function discussed above, there are many studies of mouse models of sitosterolemia, where ablation of ABCG5/G8 is able to recapitulate to a large extent the phenotypic effects. These include increased fractional plant sterol absorption, leading to highly elevated plasma and hepatic plant sterol levels (Yu *et al.*, 2005), as well as abrogated biliary cholesterol secretion. Intriguingly, such studies have also highlighted a dependence of ABCG5 and ABCG8 on other members of the ABC transporter family – specifically ABCB4 – in the biliary export of cholesterol (Langheim *et al.*, 2005), in a possible parallel to the functional synergy of ABCG1 with ABCA1.

Single nucleotide polymorphisms (SNPs)

Among the most clinically significant information on ABCG proteins that is presently needed is an understanding of the SNPs that influence both the handling of endogenous substrates and, in the case of ABCG2, the absorption and elimination of pharmaceutical agents. For ABCG2 a large body of literature is building up, evidencing not only the frequency and geographical distribution of polymorphisms but also their effect on transport of test compounds in *in vitro* assays (Tamura *et al.*, 2007). The effects *in vivo* are rather less well understood, with the exception of the SNP rs2231142 (resulting in a Gln141Lys change). This SNP, which has an allelic frequency of approximately 30% in Asians, was identified as the most significant SNP associated with gout in genome wide association studies and inferred that ABCG2 could transport uric acid, which has subsequently been confirmed (Dehghan *et al.*, 2008; Woodward *et al.*, 2009). Interestingly, previous analysis had suggested that the Gln141Lys variant of ABCG2 was only subtly different in terms of export of chemotherapeutic drugs compared with wild-type ABCG2 (Tamura *et al.*, 2007), which emphasizes that studies on the impact of SNPs on ABCG2 function have to be taken

into the context of the extreme diversity of transport substrates.

For ABCG5/G8 the elevations in plasma sterol levels (see Pathology and Clinical Significance), have led to investigations to determine whether there is any association with changes in lipoprotein profiles other than those seen in sitosterolemia. The most powerful of these are genome wide association studies on subjects in the Framingham Heart Study (*c*. 6000 participants) and those analysed as part of the European Network for Genetic and Genomic Epidemiology (*c*. 20 000 individuals) (Aulchenko *et al*., 2009; Damiani *et al*., 2010). The former identified a single locus effect of SNP rs4245791 (in intron 3 of the ABCG8) on total cholesterol levels, while the latter identified rs6756629 (resulting in an Arg50Cys change in ABCG5) as being associated with both raised total and low density lipoprotein-cholesterol. This association has important long-term consequences in recognizing a potential role for ABCG5/8 as markers for lipoprotein levels and subsequently coronary heart disease. Somewhat smaller-scale studies investigating genetic markers for gallstone formation have also identified SNPs in ABCG8 and ABCG5 as being independent factors associated with greater risk of gallstone formation (Grunhage *et al*., 2007; Kuo *et al*., 2008). For ABCG1 two small-scale reports have indicated associations between SNPs in the ABCG1 promoter and an increased risk of coronary disease, in both cases without showing any changes in plasma HDL levels (Schou *et al*., 2008; Furuyama *et al*., 2009). Further larger-scale studies are required to confirm these associations.

Pathology & clinical significance

In addition to the role in gout (see above), ABCG2 is central to the absorption and elimination of pharmaceutical agents. There are two main issues to consider relating to the ability of ABCG2 to transport multiple, chemically and structurally different drugs. First, the increased expression and activity of the transporter limits the intracellular accumulation of cytotoxic agents and hence it plays an important role in multidrug resistance (MDR) to chemotherapy. The most consistent data supports a role for ABCG2 in drug resistance in acute myeloid leukaemia, where expression correlates strongly with multiple prognostic indicators (progression free survival, disease free survival and remission rate;, e.g. (Benderra *et al*., 2004). The importance of ABCG2 in other haematological malignancies is also becoming established with recent evidence associating ABCG2 expression with both risk and prognosis for non-Hodgkin lymphoma (Kim *et al*., 2009). The precise clinical significance of ABCG2 in solid tumours remains incompletely understood. However, as discussed above, ABCG2 is able to interact with numerous front-line drugs employed in cancer care including anthracyclines (Clark *et al*., 2006), irinotecan, tyrosine kinase inhibitors (Ozvegy-Laczka *et al*., 2004), and anti-folates (Volk and Schneider, 2003), and thus we can expect more data to be forthcoming on its role in cancer MDR, as has been the case with the full-length ABC transporters ABCB1 and ABCC1.

Second, many other commonly prescribed drugs are transported by ABCG2 (Table 2), including antivirals, antibiotics, statins and calcium channel blockers, and their efficacy and the extent of side effects can be affected by common genetic variations in ABCG2. Data concerning the role of ABCG2 in limiting the absorption or facilitating the excretion of pharmaceutical drugs are widespread, and once again there is evidence that polymorphisms will impact upon clinical management (Sparreboom *et al*., 2005). These two activities of ABCG2 have led to its recognition by the International Transporter Consortium in their recent commentaries on the importance of transporters in pharmacotherapy (Giacomini *et al*., 2010).

Mutants in ABCG5 and G8 underlie sitosterolemia (OMIM 210250; phytosterolemia), an extremely rare recessively inherited disorder of sterol metabolism, first recorded in the mid 1970s (Bhattacharya and Connor, 1974). The disease is marked by high plasma concentrations of plant sterols [sitosterol and campesterol may reach 10- to 100-fold increased levels (Salen *et al*., 1985)], which are normally effectively cleared from the body. The physiological effects of this dysregulation in intestinal uptake and reduced biliary clearance of these dietary compounds manifests additionally as hyper-absorption of animal sterols from the diet, mildly raised plasma cholesterol levels, and the consequent clinical symptoms typical of hypercholesterolemia (xanthoma, coronary artery disease) (Bhattacharya and Connor, 1974; Berge *et al*., 2000). Genetics studies have identified individuals with sitosterolemia carrying mutations in either ABCG5 or ABCG8 but as yet, not in both transporters simultaneously.

While ABCG5/G8 mutations are well documented in patients with elevated plasma plant sterols, there are in contrast no known reports of ABCG1- or ABCG4-null human subjects. Unlike ABCA1, where absence of the transporter leads to Tangiers disease (a lipid disorder characterized with extensive atherosclerosis), compromised ABCG1 activity may not present with an abnormal plasma lipid profile. Of potential further importance are several reports that ABCG1 mRNA and protein expression are depressed in monocyte-macrophages from subjects with Type 2 diabetes mellitus and in alveolar macrophages from patients suffering from pulmonary alveolar proteinosis, a condition in which lung macrophage surfactant clearance is defective (Thomassen *et al*., 2007). How reduced ABCG1 levels are associated with these diseases remains to be fully elucidated, although a dysregulation of the ABCA1/ABCG1 dependence is an interesting lead to investigations.

ABCG transporter therapy

The role of ABCG proteins in human physiology and disease clearly suggests that modulating their expression and/or activity could be clinically desirable. Although there are no drugs on the market aimed at increasing ABCG1 function, there are classes of compounds under development for their anti-atherogenic potential that work via up-regulation of ABCG1-mediated cholesterol export from peripheral cells. LXR agonists, aimed at increasing expression of both ABCA1 and ABCG1, have been under development for some time but have been unsuccessful due to undesirable lipogenic side effects. Currently available LXR agonists target LXR-alpha as well as LXR-beta. LXR-alpha is the predominant subtype expressed in the liver, and an undesirable target of LXR

agonists as activation leads to increased triglyceride and non-HDL cholesterol synthesis. Future development of LXR agonists is focussing on gene or tissue selective LXR modulators or compounds with limited activation of LXR-alpha. Another class of anti-atherogenic drugs currently under investigation are the cholesterol ester transport protein inhibitors. Although the first of these, Torcetrapib®, was unsuccessful due to off-target effects [(Barter, 2009), the second wave (Anacetrapib® and Dalcetrapib®)] is currently being tested in clinical trials while further potential compounds are under development. Cholesterol ester transport protein inhibition modifies the size and composition of HDL, and it has been shown that the mechanism by which this altered HDL induces cholesterol export from macrophages is largely dependent on their expression of ABCG1 (Matsuura *et al.*, 2006). Also under investigation is the use of reconstituted HDLs, which has been shown to be athero-protective in human trials (Tardif *et al.*, 2007; Shaw *et al.*, 2008). A recent study indicated that cholesterol mobilization from macrophages to reconstituted HDLs is primarily mediated by ABCG1 (Cuchel *et al.*, 2010). Considering the multitude of therapies aimed at increasing macrophage cholesterol export via ABCG1, the basic workings of this transporter, in terms of regulation, as well as the importance of isoforms and species differences need to be better understood. In terms of treatment strategies for ABCG5/G8 related conditions, the therapy is not targeted at the ABCG5/8 proteins as sitosterolemia can be controlled to a large degree by dietary modification. Bile acid resins and sterol-absorption inhibitors (ezetimibe) are also effective in further reducing plasma plant sterol levels (Salen *et al.*, 2004).

In the case of ABCG2, the need is of course for inhibitors instead of agonists. Highly specific ABCG2 inhibition by the fungal compound ABCG2 fumitremorgin C has been demonstrated but is too toxic for *in vivo* use (Table 2). Less neurotoxic derivatives (Ko143) have been identified but have not reached clinical trials. Inhibitors of other MDR-type ABC transporters have long been sought to address the clinical problem of chemotherapy failure. At least one of the so-called third generation inhibitors of ABCB1 has also shown itself to be an inhibitor of ABCG2 at sub-micromolar concentrations *in vitro*, namely elacridar/GF120918, and phase I clinical trials of elacridar as an ABCG2 inhibitor have been reported (Kuppens *et al.*, 2007), but we remain far short of an ability to reverse ABCG2-mediated MDR in the clinic.

Conclusion

Our understanding of the role of ABCG proteins in human physiology and disease is still somewhat superficial, and there is much more we need to address. One important question in terms of this review is that the identification of substrates and their relative affinities, as well as clinical modulators and inhibitors of action remains incomplete. If the picture for ABCG1, G4, G5 and G8 is anything like as complex as the situation for ABCG2 then this in itself could be a substantial undertaking. The interaction of ABCG proteins with other membrane-associated proteins is another important area of research, as not only will this affect transporter activity, but it may well impact on the transporter substrate profile. As we have outlined above, genomic data are already providing some understanding of how SNPs in ABCG proteins may contribute to disease predisposition, and with the advent of human population genome sequencing projects, this will continue to assume importance. Finally, we need to be open to the prospect of multiple functions for ABCG proteins, depending on the cellular context, which is already being demonstrated in the case of ABCG2. Unravelling this further level of complexity will prove one of the major challenges ahead.

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Conflict of interest

The authors declare no conflict of interest.

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