ABCG2

Subjects: Biochemistry & Molecular Biology
Contributor: László Homolya

The ABCG2 (also named breast cancer resistance protein—BCRP, or mitoxantrone resistance protein—MXR) is an integral membrane protein belonging to the ABC (ATP-binding cassette) protein superfamily. ABCG2 is an active transporter utilizing the energy of ATP binding and hydrolysis to translocate various substrate molecules across the plasma membrane from the cells to the extracellular space. Its transported substrates include endobiotics (endogenous substances), such as uric acid, as well as xenobiotics, such as anti-cancer drugs. ABCG2 plays a pivotal role in uric acid clearance; thus, its malfunction may lead to hyperuricemia and gout. On the other hand, ABCG2 residing in various barrier tissues is involved in the innate defense mechanisms of the body, influencing the absorption, distribution, excretion of potentially toxic endo- and exogenous compounds.

Keywords: ABC (ATP-binding cassette) transporters ; multidrug resistance ; transport ; trafficking ; urate ; mutations ; polymorphisms

1. Introduction

The ABCG2 protein is a member of the ABC (ATP-binding cassette) protein superfamily. A distinguishing hallmark of ABC proteins is the presence of Walker A, Walker B, and the so-called ABC signature (typically LSGGQ) motifs in their sequences. The members of this large protein family are present in all living organisms, ranging from prokaryotes through fungi, plants, invertebrates to vertebrates. The design of ATP-binding fold and its connection to transport mechanisms seem evolutionarily beneficial, as they have been conserved through evolution [1]. In the human genome, there are 48 genes encoding ABC proteins, which are classified into seven subfamilies (denoted from A to G) primarily on the basis of sequence homology. Most of the human ABC proteins are membrane proteins mediating translocation of substances across biological membranes using the energy of ATP binding and hydrolysis. There are some peculiar members of the family, like the regulatory ABC proteins, exemplified by the sulfonylurea receptors (SUR1/ABCC8 and SUR2/ABCC9), which control the function of other membrane proteins; or the cystic fibrosis transmembrane regulator (CFTR/ABCC7), which is an ion channel facilitating downhill chloride transport across the membrane.

Some human ABC proteins are specialized in the transport of one or a limited number of substrates. For example, MDR3/ABCB4 mediates phosphatidylcholine transport in the canalicular membrane of hepatocytes. In contrast, MDR1 (P-glycoprotein, ABCB1) is rather promiscuous, transporting a large variety of unrelated molecules. Membrane transporter proteins with broad substrate recognition may confer resistance in cells to multiple drugs, i.e., causing cross-resistance in tumor cells. These transporters are called multidrug resistance (MDR) proteins, although they also play a pivotal role at important physiological tissue barriers controlling the uptake and excretion of endo- and xenobiotics. In humans, there are multidrug transporters from the ABCB, the ABCC, and the ABCG subfamilies. These MDR proteins with their broad and partially overlapping substrate recognition, as well as with their tissue- and cell type-specific expression constitute a complex physiological network, called the chemoimmunity system, which is an essential part of the innate defense system against harmful substances [2].

ABCG2 was originally identified as a multidrug transporter in multidrug-resistant cancer cell lines, in which none of the two MDR proteins known at that time (MDR1/ABCB1 and MRP1/ABCC1) was expressed [3][4]. In drug-selected cells and certain tumors, ABCG2 is massively overexpressed and may contribute to the poor clinical outcome of these tumors [5][6]. Its physiological presence in normal tissues has also been demonstrated—first in placenta [7], and subsequently in a large variety of other tissues. The wide-ranging but still specific tissue distribution, combined with the broad substrate recognition, makes ABCG2 an essential element of the chemoimmunity network.

2. Architecture of ABCG2

ABCG2 belongs to the ABCG subfamily, the members of which, in addition to sequence homology, exhibit considerable structural similarities. To our recent knowledge, the minimal structure of a functional ABC transporter is composed of two cytoplasmic nucleotide-binding domains (NBDs) and two transmembrane domains (TMDs). The usual arrangement of domains in the core protein is as follows: TMD1-NBD1-TMD2-NBD2. Contrary to the canonical ABC transporters, members of the ABCG subfamily possess only one NBD and one TMD; thus, they are called half-transporters. Moreover, the domain order in ABCG proteins is reversed, i.e., NBD is localized N-terminally to TMD. This reverse domain arrangement could be one of reasons for the sensitivity of ABCG2 to tagging at the C-terminus [8], contrary to many other ABC transporters, which can regularly be tagged C-terminally. To form a functional complex, ABCG proteins, like other ABC half-transporters, assemble into either homo- or heterodimers. While ABCG2 solely forms homodimers, ABCG5 and ABCG8 are obligate heterodimers. In contrast, two other members of the subfamily, ABCG1 and ABCG4 can form both homo- and heterodimers [9].

Membrane topology models of ABCG2 suggest six membrane-spanning helices, a relatively short C-terminal tail, and short loops between the transmembrane helices (TMHs) except for the last extracellular loop (EL3) between TMH5 and TMH6 [10]. N-glycosylation on asparagine 596 located in the EL3 loop has also been demonstrated [11][12]. Although initial reports suggested that glycosylation at N596 was not essential for proper expression, localization, and function, subsequent studies demonstrated N-glycosylation to be an important checkpoint determining the stability and intracellular trafficking of the transporter [13][14]. There are twelve cysteine residues in ABCG2, but only three of them are positioned in an oxidative milieu, and thus capable of forming disulfide bonds. All three of these cysteines are located in the EL3 loop, and while an intramolecular disulfide bond is established between C592 and C608, an intermolecular disulfide bridge is formed between the two halves of the homodimer at C603. Whereas the latter disulfide bond is not required for proper trafficking and function of ABCG2 [15][16][17], the C592-C608 intramolecular disulfide bond represents another critical checkpoint for protein folding and trafficking [14][17].

Although the X-ray structures of isolated NBDs have been available since the late nineties, the first high-resolution structures of full-length ABC transporters were only published in 2006 and 2007 [18][19]. In the last decade, the spread of cryogenic electron microscopy (cryo-EM) and the substantial progress in crystallography have given a boost to our understanding of ABC protein structures. However, homology modeling was not quite applicable to ABCG proteins, as the members of this family are rather distinct from other ‘classical’ ABC transporters, such as the P-glycoprotein (MDR1/ABCB1) or the CFTR/ABCC7. The appearance of the first high-resolution structure of an ABCG protein, i.e., that of the heterodimeric sterol transporter ABCG5/ABCG8, was therefore a breakthrough [20], fueling extensive homology modeling of ABCG2 [21][22][23]. Subsequently, several ABCG2 structures based on cryo-EM analyses have been published [24][25][26][27]. The structural characteristics of ACBG2, obtained from these cryo-EM studies and from parallel molecular dynamic stimulations, are recently reviewed in [28][29].

There are some distinguishing structural features in the ACBG2 as compared to the full-length ABC transporters. In general, the structure of ABCG2 is more compact, the NBDs are positioned close to the TMDs. A similar compact arrangement was observed for the ABCG5/ABCG8 crystal structure [20], which resembles the architecture of BtuCD-like bacterial importers, rather than that of MDR1-like transporters. This originates from the relatively short transmembrane helices, possessing no cytosolic extension unlike the helices in the classical ABC proteins, which create a sort of spacer between the TMDs and NBDs. In the MDR1-like proteins, two of the elongated four pairs of helices cross over and bind to the opposite NBD, while the other two pairs interact with the ipsilateral NBD. The interfacings to NBDs are realized by small, so-called coupling helices at the cytosolic tip of the elongated TMHs [18]. In contrast, there is only one coupling helix in each half of the ABCG2 dimer (between TMH2 and TMH3), which does not cross over to the other half. However, an amphipathic helix, called the connecting helix, linked to only TMH1 and reclining against the membrane bilayer, provides an additional TMD-NBD interface in ABCG2.

Another distinct feature of the structure of ABCG2, as well as of ABCG5-ABCG5, is the relatively closed conformation in the absence of ATP. In the classical ABC transporters in this ‘apo’ form (without ATP), the NBDs are located far from one another, and consequently, the intracellular parts of TMHs also remain apart, forming a large central cavity, the main substrate-binding pocket, which is widely open to the cytoplasm [18]. The presence of a similar cavity at the cytoplasmic side of ABCG2 (cavity 1) has been reported by cryo-EM studies using an anti-ABCG2 antibody to reduce flexibility in the structure [24][25][26]. Nevertheless, the NBDs, and consequently the intracellular parts of TMHs, are closer to one another in ABCG2 than in MDR-like transporters, resulting in a more compact structure even in the absence of ATP. Residues in this central cavity were shown to be essential not only for transport function but also for biogenesis [23]. Interestingly, a study using no anti-ABCG2 antibodies for structure stabilization reported the lack of cavity 1 [27]. An additional prominent feature in the inward-facing structure (apo form) of ABCG2 is the hydrophobic di-leucine valve (L554 and L555) separating the

central substrate-binding pocket from an additional cavity (cavity 2) located toward the extracellular part of ABCG2 [24][25]. Experiments supplemented by molecular dynamic simulations demonstrated an essential role for this di-leucine plug in the transport function [30]. Putting together the different structures in the absence and presence of ATP and/or substrates, MDR1-like proteins seem to alternate between a widely open inward-facing and a fairly open outward-facing conformations, whereas the translocation of substrates through ABCG2 via cavities 1 and 2 rather involves a peristaltic-like movement.

With regard to the NBDs, both sequences and structures are fairly conserved. The two composite ATP-binding pockets are constituted by two separate NBDs in a head-to-tail orientation, i.e., one ATP molecule binds to the Walker A and B motifs of one NBD and to the ABC signature sequence of the other NBD. Unlike in full-length ABC transporters, the cytoplasmic part of the homodimeric ABCG2 is composed of two identical halves, but otherwise the ABC-folds in ABCG2 are structurally similar to that of the classical ABC transporters. It is worth noting that a phenylalanine at position 142 in ABCG2 interacts with the connecting helix, representing a key residue in TMD-NBD interface assembly and a critical checkpoint for protein folding and function [31][32]. Interestingly, this amino acid is analogous to F508 in CFTR/ABCC7, the mutation of which is responsible for diminished trafficking of CFTR, and ultimately the cystic fibrosis (CF) phenotype.

3. The Physiological Functions of ABCG2, and Its Role in Multidrug Resistance

3.1. The Physiological Roles of ABCG2

As mentioned previously, ABCG2 is overexpressed in drug-resistant cell lines and tumors. Habitually, it is expressed at a relatively high level in cell types located at the entry and exit boundaries of the body, as well as in barrier tissues at the borders of sanctuary sites [33][34]. These include the epithelial cells of the gastrointestinal track, especially in small intestine enterocytes [35], the kidney tubular epithelial cells [36], hepatocytes [33], placental syncytiotrophoblasts [37], mammary alveolar epithelial cells (a part of the blood-milk barrier) [38], and brain capillary endothelial cells (a key element of the blood-brain barrier) [39][40]. In these polarized epithelial and endothelial cells, ABCG2 is localized to the apical plasma membrane domain. In addition to these cells constituting tissue barriers, ABCG2 is also expressed in various types of stem cells including hematopoietic stem cells [41], pluripotent stem cells [42][43], and cancer stem cells [44][45][46][47]. Interestingly, ABCG2 is also present in the membrane of red blood cells (RBCs) [33][48][49].

As is typical of a multidrug transporter, ABCG2 recognizes a vast variety of compounds as transported substrate molecules. These include uric acid in the first place, but also various endogenous conjugated hormones and metabolites, several hydrophobic and amphipathic drugs, as well as their conjugates [50][51][52]. This promiscuity and the tissue distribution detailed above delineate the physiological function of this transporter. In general, ABCG2—depending on its location—restricts the uptake or facilitates the excretion of potentially toxic or unwanted substances. Specifically, in the brain capillaries, ABCG2 restricts the passage of substances through the blood-brain barrier, whereas in the placenta, it protects the fetus from maternally derived toxins. For instance, ABCG2 restricts the maternal-fetal transfer of bile acids, which is especially important in expecting mothers with intrahepatic cholestasis of pregnancy, a frequent liver disease leading to augmented serum levels of bile acids [53][54]. In the small intestine, ABCG2 controls the absorption of various molecules and participates in extra-renal clearance of uric acid; in the kidney proximal tubules, it contributes to the elimination of unwanted toxins and metabolites, including uric acid. Impaired ABCG2-mediated urate transport may lead to gout or hyperuricemia, therefore, specific mutations and polymorphisms in ABCG2 are genetic risk factors for these conditions [36][55][56][57]. Interestingly, a recent study reported unequal contribution of ABCG2 to renal and extra-renal clearance of uric acid [57]. In mammary alveolar epithelial cells, this transporter influences the milk composition. Endogenous substrates transported by ABCG2 through the blood-milk barrier include riboflavin (vitamin B_2) and bile acids [58][59]. Certainly, vigilance is required for breast-feeding mothers, as various medications can be transported by ABCG2 into the milk [38][60]. The relevance of the Abcg2-mediated drug transport for the dairy industries is also self-evident [61][62].

In these physiological boundaries, ABCG2 accomplishes this ‘bouncer duty’ in a coordinated fashion together with the other MDR proteins, MDR1/ABCB1 and MRP1/ABCC1, exploiting their partially overlapping substrate recognition and specific subcellular localization. In polarized epithelial cells, ABCG2 is localized to the apical membrane ipsilaterally to MDR1/ABCB1 and contralaterally to MRP1/ABCC1, whereas in cerebral endothelial cells, all three major MDR proteins reside at the same side, i.e., the apical membrane [63]. Accordingly, ABCG2 along with other MDR proteins potentially alters the absorption, distribution, and excretion, as well as, consequently, the metabolism and toxicity (ADME-Tox properties) of pharmaceutical drugs. Especially important is the potential contribution of these transporters to drug–drug interactions, since modification of one (or more) of the MDR proteins by a drug may greatly influence the

pharmacokinetics of another one. Therefore, the examination of drug interactions with MDR proteins, including ABCG2, is a requirement in preclinical drug development [52][64][65]. Interestingly, in mammary epithelial cells, the apically localized ABCG2 and the basolateral MRP4/ABCC4 counteract one another in bile acid transport [59].

The physiological role of ABCG2 in red blood cells and stem cells is enigmatic to some extent. Since phototoxic porphyrins, such as the plant-derived pheophoride A and the heme precursor protoporphyrin IX (PPIX), are noted substrates of ABCG2, its expression in the erythroid precursor cells and in mature RBCs may indicate its involvement in heme metabolism [49][66][67]. It is worth noting, however, that numerous membrane proteins without known function in RBCs are present in their membrane, i.e., the sterol transporter ABCA1 (http://rbcc.hegelab.org/, accessed on 9 February 2021) [68][69]. It is plausible that many of these membrane proteins can be just remnants from previous stages of cell differentiation and maturation. In various stem cell types, a protective role similar to that observed at the border of sanctuary sites has been proposed for ABCG2 [41][42][43]. Stem cells are poised between self-renewal and differentiation, and are thus exceptionally sensitive to environmental factors. ABCG2 can contribute to the stem cells' self-protective mechanisms. The presence of the transporter may, however, backfire in cancer stem cells, as they can provide tumors with drug-resistant cell populations.

3.2. The Involment of ABCG2 in Multidrug Resistance of Cancer

Beyond its physiological roles, ABCG2 has been implicated in cancer multidrug resistance (recently reviewed in [6]). A large variety of chemotherapeutic agents has been identified as ABCG2 substrates. First, the anti-cancer drug mitoxantrone has been demonstrated to be exported by ABCG2, thus reducing its intracellular accumulation [3][70][71][72]. Interestingly, a kinetic analysis indicated that mitoxantrone is extruded by ABCG2 not from the cytosol but directly from the plasma membrane, where the drug accumulates [73]. Other anti-cancer drugs identified as ABCG2 substrates include flavopiridol [72][74], methotrexate [75], topotecan, and irinotecan [76][77]. In addition, several prominent tyrosine kinase inhibitors (TKIs) used in chemotherapies, such as gefitinib [78][79][80], imatinib [80][81][82], sunitinib [83], and nilotinib [82][84], were proven to be transported by ABCG2. The anti-cancer agents doxorubicin and daunorubicin have also been reported as ABCG2 substrates [3][71][74], but eventually it was revealed that these drugs are transported only by the R482G ABCG2 variant [2][85].

Expression of ABCG2 in tumors often correlates with poor prognosis, especially in hematopoietic malignancies, such as acute myeloid leukemia [86], but also in solid tumors, including diffuse large B-cell lymphoma [87]. However, clinical data are often conflicting like in the case of acute lymphocytic leukemia [88][89][90], or of breast carcinoma [91][92][93]. Several other studies demonstrated correlation between ABCG2 expression and response to chemotherapy, even to drugs, which are not ABCG2 substrates. These inconsistencies can originate from the modulatory effect of other drug resistance mechanisms, most evidently the presence of other MDR proteins. In addition, the methods employed to determine ABCG2 expression could be dubious, originating from the potential cross-reactivity of applied antibodies, or from the fact that mRNA levels of membrane proteins often do not correlate with the protein levels. The genetic background of patients could give an extra hue to these clinical studies as mutations and polymorphisms may alter the input of ABCG2 into the clinical outcome or the response to various drugs; therefore, proper stratification of patients is crucial for these analyses. The role of ABCG2 in tumors has been implicated, but its actual contribution to the clinical multidrug resistance is still unclear [5][6].

References

1. Ogasawara, F.; Kodan, A.; Ueda, K. ABC proteins in evolution. FEBS Lett. 2020, 594, 3876–3881.
2. Sarkadi, B.; Homolya, L.; Szakács, G.; Váradi, A. Human multidrug resistance ABCB and ABCG transporters: Participation in a chemoimmunity defense system. Physiol. Rev. 2006, 86, 1179–1236.
3. Doyle, L.A.; Yang, W.; Abruzzo, L.V.; Krogmann, T.; Gao, Y.; Rishi, A.K.; Ross, D.D. A multidrug resistance transporter from human MCF-7 breast cancer cells. Proc. Natl. Acad. Sci. USA 1998, 95, 15665–15670.
4. Litman, T.; Brangi, M.; Hudson, E.; Fetsch, P.; Abati, A.; Ross, D.D.; Miyake, K.; Resau, J.H.; Bates, S.E. The multi-drug-resistant phenotype associated with overexpression of the new ABC half-transporter, MXR (ABCG2). J. Cell. Sci. 2000, 113, 2011–2021.
5. Robey, R.W.; Polgar, O.; Deeken, J.; To, K.W.; Bates, S.E. ABCG2: Determining its relevance in clinical drug resistance. Cancer Metastasis Rev. 2007, 26, 39–57.
6. Robey, R.W.; Pluchino, K.M.; Hall, M.D.; Fojo, A.T.; Bates, S.E.; Gottesman, M.M. Revisiting the role of ABC transporters in multidrug-resistant cancer. Nat. Rev. Cancer 2018, 18, 452–464.

7. Allikmets, R.; Schriml, L.M.; Hutchinson, A.; Romano-Spica, V.; Dean, M. A human placenta-specific ATP-binding cassette gene (ABCP) on chromosome 4q22 that is involved in multidrug resistance. Cancer Res. 1998, 58, 5337–5339.
8. Orbán, T.I.; Seres, L.; Özvegy-Laczka, C.; Elkind, N.B.; Sarkadi, B.; Homolya, L. Combined localization and real-time functional studies using a GFP-tagged ABCG2 multidrug transporter. Biochem. Biophys. Res. Commun. 2008, 367, 667–673.
9. Hegyi, Z.; Homolya, L. Functional cooperativity between ABCG4 and ABCG1 isoforms. PLoS ONE 2016, 11, e0156516.
10. McDevitt, C.A.; Collins, R.; Kerr, I.D.; Callaghan, R. Purification and structural analyses of ABCG2. Adv. Drug Deliv. Rev. 2009, 61, 57–65.
11. Diop, N.K.; Hrycyna, C.A. N-linked glycosylation of the human ABC transporter ABCG2 on asparagine 596 Is not essential for expression, transport activity, or trafficking to the plasma membrane. Biochemistry 2005, 44, 5420–5429.
12. Mohrmann, K.; Van Eijndhoven, M.A.J.; Schinkel, A.H.; Schellens, J.H.M. Absence of N-linked glycosylation does not affect plasma membrane localization of breast cancer resistance protein (BCRP/ABCG2). Cancer Chemother. Pharmacol. 2005, 56, 344–350.
13. Nakagawa, H.; Wakabayashi-Nakao, K.; Tamura, A.; Toyoda, Y.; Koshiba, S.; Ishikawa, T. Disruption of N-linked glycosylation enhances ubiquitin-mediated proteasomal degradation of the human ATP-binding cassette transporter ABCG2. FEBS J. 2009, 276, 7237–7252.
14. Wakabayashi-Nakao, K.; Tamura, A.; Furukawa, T.; Nakagawa, H.; Ishikawa, T. Quality control of human ABCG2 protein in the endoplasmic reticulum: Ubiquitination and proteasomal degradation. Adv. Drug Deliv. Rev. 2009, 61, 66–72.
15. Henriksen, U.; Fog, J.U.; Litman, T.; Gether, U. Identification of intra- and intermolecular disulfide bridges in the multidrug resistance transporter ABCG2. J. Biol. Chem. 2005, 280, 36926–36934.
16. Kage, K.; Fujita, T.; Sugimoto, Y. Role of Cys-603 in dimer/oligomer formation of the breast cancer resistance protein BCRP/ABCG2. Cancer Sci. 2005, 96, 866–872.
17. Wakabayashi, K.; Nakagawa, H.; Adachi, T.; Kii, I.; Kobatake, E.; Kudo, A.; Ishikawa, T. Identification of cysteine residues critically involved in homodimer formation and protein expression of human ATP-binding cassette transporter ABCG2: A new approach using the flp recombinase system. J. Exp. Ther. Oncol. 2006, 5, 205–222.
18. Dawson, R.J.P.; Locher, K.P. Structure of a bacterial multidrug ABC transporter. Nat. Cell Biol. 2006, 443, 180–185.
19. Ward, A.; Reyes, C.L.; Yu, J.; Roth, C.B.; Chang, G. Flexibility in the ABC transporter MsbA: Alternating access with a twist. Proc. Natl. Acad. Sci. USA 2007, 104, 19005–19010.
20. Lee, J.-Y.; Kinch, L.N.; Borek, D.M.; Wang, J.; Wang, J.; Urbatsch, I.L.; Xie, X.-S.; Grishin, N.V.; Cohen, J.C.; Otwinowski, Z.; et al. Crystal structure of the human sterol transporter ABCG5/ABCG8. Nat. Cell Biol. 2016, 533, 561–564.
21. László, L.; Sarkadi, B.; Hegedűs, T. Jump into a new fold—A homology based model for the ABCG2/BCRP multidrug transporter. PLoS ONE 2016, 11, e0164426.
22. Ferreira, R.J.; Bonito, C.A.; Cordeiro, M.N.D.S.; Ferreira, M.-J.U.; Dos Santos, D.J.V.A. Structure-function relationships in ABCG2: Insights from molecular dynamics simulations and molecular docking studies. Sci. Rep. 2017, 7, 15534.
23. Khunweeraphong, N.; Stockner, T.; Kuchler, K. The structure of the human ABC transporter ABCG2 reveals a novel mechanism for drug extrusion. Sci. Rep. 2017, 7, 1–15.
24. Taylor, N.M.I.; Manolaridis, I.; Jackson, S.M.; Kowal, J.; Stahlberg, H.; Locher, K.P. Structure of the human multidrug transporter ABCG2. Nat. Cell Biol. 2017, 546, 504–509.
25. Manolaridis, I.; Jackson, S.M.; Taylor, N.M.I.; Kowal, J.; Stahlberg, H.; Locher, K.P. Cryo-EM structures of a human ABCG2 mutant trapped in ATP-bound and substrate-bound states. Nat. Cell Biol. 2018, 563, 426–430.
26. Jackson, S.M.; Manolaridis, I.; Kowal, J.; Zechner, M.; Taylor, N.M.I.; Bause, M.; Bauer, S.; Bartholomaeus, R.; Bernhardt, G.; Koenig, B.; et al. Structural basis of small-molecule inhibition of human multidrug transporter ABCG2. Nat. Struct. Mol. Biol. 2018, 25, 333–340.
27. Orlando, B.J.; Liao, M. ABCG2 transports anticancer drugs via a closed-to-open switch. Nat. Commun. 2020, 11, 1–11.
28. Eckenstaler, R.; Benndorf, R.A. 3D structure of the transporter ABCG2—What's new? Br. J. Pharmacol. 2020, 177, 1485–1496.
29. Sarkadi, B.; Homolya, L.; Hegedűs, T. The ABCG2/BCRP transporter and its variants—From structure to pathology. FEBS Lett. 2020, 594, 4012–4034.

30. Khunweeraphong, N.; Szöllősi, D.; Stockner, T.; Kuchler, K. The ABCG2 multidrug transporter is a pump gated by a valve and an extracellular lid. Nat. Commun. 2019, 10, 1–14.

31. Sarankó, H.; Tordai, H.; Telbisz, A.; Ozvegy-Laczka, C.; Erdös, G.; Sarkadi, B.; Hegedűs, T. Effects of the gout-causing Q141K polymorphism and a CFTR ΔF508 mimicking mutation on the processing and stability of the ABCG2 protein. Biochem. Biophys. Res. Commun. 2013, 437, 140–145.

32. Woodward, O.M.; Tukaye, D.N.; Cui, J.; Greenwell, P.; Constantoulakis, L.M.; Parker, B.S.; Rao, A.; Köttgen, M.; Maloney, P.C.; Guggino, W.B. Gout-causing Q141K mutation in ABCG2 leads to instability of the nucleotide-binding domain and can be corrected with small molecules. Proc. Natl. Acad. Sci. USA 2013, 110, 5223–5228.

33. Maliepaard, M.; Scheffer, G.L.; Faneyte, I.F.; Van Gastelen, M.A.; Pijnenborg, A.C.; Schinkel, A.H.; Van De Vijver, M.J.; Scheper, R.J.; Schellens, J.H. Subcellular localization and distribution of the breast cancer resistance protein transporter in normal human tissues. Cancer Res. 2001, 61, 3458–3464.

34. Leslie, E.M.; Deeley, R.G.; Cole, S.P. Multidrug resistance proteins: Role of P-glycoprotein, MRP1, MRP2, and BCRP (ABCG2) in tissue defense. Toxicol. Appl. Pharmacol. 2005, 204, 216–237.

35. Gutmann, H.; Hruz, P.; Zimmermann, C.; Beglinger, C.; Drewe, J. Distribution of breast cancer resistance protein (BCRP/ABCG2) mRNA expression along the human GI tract. Biochem. Pharmacol. 2005, 70, 695–699.

36. Woodward, O.M.; Köttgen, M.; Coresh, J.; Boerwinkle, E.; Guggino, W.B. Identification of a urate transporter, ABCG2, with a common functional polymorphism causing gout. Proc. Natl. Acad. Sci. USA 2009, 106, 10338–10342.

37. Mao, Q. BCRP/ABCG2 in the placenta: Expression, function and regulation. Pharm. Res. 2008, 25, 1244–1255.

38. Jonker, J.W.; Merino, G.; Musters, S.; Van Herwaarden, A.E.; Bolscher, E.; Wagenaar, E.; Mesman, E.; Dale, T.C.; Schinkel, A.H. The breast cancer resistance protein BCRP (ABCG2) concentrates drugs and carcinogenic xenotoxins into milk. Nat. Med. 2005, 11, 127–129.

39. Cooray, H.C.; Blackmore, C.G.; Maskell, L.; Barrand, M.A. Localisation of breast cancer resistance protein in microvessel endothelium of human brain. NeuroReport 2002, 13, 2059–2063.

40. Zhang, W.; Mojsilovic-Petrovic, J.; Andrade, M.F.; Zhang, H.; Ball, M.; Stanimirovic, D.B. Expression and functional characterization of ABCG2 in brain endothelial cells and vessels. FASEB J. 2003, 17, 1–24.

41. Zhou, S.; Schuetz, J.D.; Bunting, K.D.; Colapietro, A.-M.; Sampath, J.; Morris, J.J.; Lagutina, I.; Grosveld, G.C.; Osawa, M.; Nakauchi, H.; et al. 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. 2001, 7, 1028–1034.

42. Apáti, A.; Orbán, T.I.; Varga, N.; Németh, A.; Schamberger, A.; Krizsik, V.; Erdélyi-Belle, B.; Homolya, L.; Várady, G.; Padányi, R.; et al. High level functional expression of the ABCG2 multidrug transporter in undifferentiated human embryonic stem cells. Biochim. Biophys. Acta BBA Biomembr. 2008, 1778, 2700–2709.

43. Sarkadi, B.; Orbán, T.I.; Szakacs, G.; Várady, G.; Schamberger, A.; Erdei, Z.; Szebényi, K.; Homolya, L.; Apáti, A. Evaluation of ABCG2 expression in human embryonic stem cells: Crossing the same river twice? Stem Cells 2009, 28, 174–176.

44. Dean, M.; Fojo, T.; Bates, S.E. Tumour stem cells and drug resistance. Nat. Rev. Cancer 2005, 5, 275–284.

45. Ho, M.M.; Ng, A.V.; Lam, S.; Hung, J.Y. Side Population in human lung cancer cell lines and tumors is enriched with stem-like cancer cells. Cancer Res. 2007, 67, 4827–4833.

46. Ding, X.-W.; Wu, J.-H.; Jiang, C.-P. ABCG2: A potential marker of stem cells and novel target in stem cell and cancer therapy. Life Sci. 2010, 86, 631–637.

47. Borst, P. Cancer drug pan-resistance: Pumps, cancer stem cells, quiescence, epithelial to mesenchymal transition, blocked cell death pathways, persisters or what? Open Biol. 2012, 2, 120066.

48. Kasza, I.; Várady, G.; Andrikovics, H.; Koszarska, M.; Tordai, A.; Scheffer, G.L.; Németh, A.; Szakács, G.; Sarkadi, B. Expression levels of the ABCG2 multidrug transporter in human erythrocytes correspond to pharmacologically relevant genetic variations. PLoS ONE 2012, 7, e48423.

49. Zhou, S.; Zong, Y.; Ney, P.A.; Nair, G.; Stewart, C.F.; Sorrentino, B.P. Increased expression of the Abcg2 transporter during erythroid maturation plays a role in decreasing cellular protoporphyrin IX levels. Blood 2005, 105, 2571–2576.

50. Borst, P.; Elferink, R.O. Mammalian ABC transporters in health and disease. Annu. Rev. Biochem. 2002, 71, 537–592.

51. Van Herwaarden, A.E.; Schinkel, A.H. The function of breast cancer resistance protein in epithelial barriers, stem cells and milk secretion of drugs and xenotoxins. Trends Pharmacol. Sci. 2006, 27, 10–16.

52. The International Transporter Consortium. Membrane transporters in drug development. Nat. Rev. Drug Discov. 2010, 9, 215–236.

53. Blazquez, A.G.; Briz, O.; Romero, M.R.; Rosales, R.; Monte, M.J.; Vaquero, J.; Macias, R.I.R.; Cassio, R.; Marin, J.J.G. Characterization of the Role of ABCG2 as a bile acid transporter in liver and placenta. Mol. Pharmacol. 2011, 81, 273–283.

54. Blazquez, A.G.; Briz, O.; Gonzalez-Sanchez, E.; Perez, M.J.; Ghanem, C.I.; Marin, J.J. The effect of acetaminophen on the expression of BCRP in trophoblast cells impairs the placental barrier to bile acids during maternal cholestasis. Toxicol. Appl. Pharmacol. 2014, 277, 77–85.

55. Matsuo, H.; Takada, T.; Ichida, K.; Nakamura, T.; Nakayama, A.; Ikebuchi, Y.; Ito, K.; Kusanagi, Y.; Chiba, T.; Tadokoro, S.; et al. Common defects of ABCG2, a high-capacity urate exporter, cause gout: A function-based genetic analysis in a Japanese population. Sci. Transl. Med. 2009, 1, 5ra11.

56. Chen, L.; Manautou, J.E.; Rasmussen, T.P.; Zhong, X.-B. Development of precision medicine approaches based on inter-individual variability of BCRP/ABCG2. Acta Pharm. Sin. B 2019, 9, 659–674.

57. Hoque, K.M.; Dixon, E.E.; Lewis, R.M.; Allan, J.; Gamble, G.D.; Phipps-Green, A.J.; Kuhns, V.L.H.; Horne, A.M.; Stamp, L.K.; Merriman, T.R.; et al. The ABCG2 Q141K hyperuricemia and gout associated variant illuminates the physiology of human urate excretion. Nat. Commun. 2020, 11, 1–15.

58. Van Herwaarden, A.E.; Wagenaar, E.; Merino, G.; Jonker, J.W.; Rosing, H.; Beijnen, J.H.; Schinkel, A.H. Multidrug transporter ABCG2/breast cancer resistance protein secretes riboflavin (Vitamin B2) into milk. Mol. Cell. Biol. 2006, 27, 1247–1253.

59. Blazquez, A.M.G.; Macias, R.I.R.; Cives-Losada, C.; De La Iglesia, A.; Marin, J.J.G.; Monte, M.J. Lactation during cholestasis: Role of ABC proteins in bile acid traffic across the mammary gland. Sci. Rep. 2017, 7, 1–11.

60. Vlaming, M.L.; Lagas, J.S.; Schinkel, A.H. Physiological and pharmacological roles of ABCG2 (BCRP): Recent findings in Abcg2 knockout mice. Adv. Drug Deliv. Rev. 2009, 61, 14–25.

61. Lindner, S.; Halwachs, S.; Wassermann, L.; Honscha, W. Expression and subcellular localization of efflux transporter ABCG2/BCRP in important tissue barriers of lactating dairy cows, sheep and goats. J. Vet. Pharmacol. Ther. 2013, 36, 562–570.

62. Mahnke, H.; Ballent, M.; Baumann, S.; Imperiale, F.; Von Bergen, M.; Lanusse, C.; Lifschitz, A.L.; Honscha, W.; Halwachs, S. The ABCG2 efflux transporter in the mammary gland mediates veterinary drug secretion across the blood-milk barrier into milk of dairy cows. Drug Metab. Dispos. 2016, 44, 700–708.

63. Bakos, E.; Homolya, L. Portrait of multifaceted transporter, the multidrug resistance-associated protein 1 (MRP1/ABCC1). Pflügers Arch. Eur. J. Physiol. 2006, 453, 621–641.

64. Giacomini, K.M.; Balimane, P.V.; Cho, S.K.; Eadon, M.; Edeki, T.; Hillgren, K.M.; Huang, S.-M.; Sugiyama, Y.; Weitz, D.; Wen, Y.; et al. International transporter consortium commentary on clinically important transporter polymorphisms. Clin. Pharmacol. Ther. 2013, 94, 23–26.

65. Prueksaritanont, T.; Chu, X.; Gibson, C.; Cui, D.; Yee, K.L.; Ballard, J.; Cabalu, T.; Hochman, J. Drug–drug interaction studies: Regulatory guidance and an industry perspective. AAPS J. 2013, 15, 629–645.

66. Jonker, J.W.; Buitelaar, M.; Wagenaar, E.; Van Der Valk, M.A.; Scheffer, G.L.; Scheper, R.J.; Plösch, T.; Kuipers, F.; Elferink, R.P.J.O.; Rosing, H.; et al. Nonlinear partial differential equations and applications: The breast cancer resistance protein protects against a major chlorophyll-derived dietary phototoxin and protoporphyria. Proc. Natl. Acad. Sci. USA 2002, 99, 15649–15654.

67. Wang, P.; Sachar, M.; Lu, J.; Shehu, A.I.; Zhu, J.; Chen, J.; Liu, K.; Anderson, K.E.; Xie, W.; Gonzalez, F.J.; et al. The essential role of the transporter ABCG2 in the pathophysiology of erythropoietic protoporphyria. Sci. Adv. 2019, 5, eaaw6127.

68. Várady, G.; Szabó, E.; Fehér, A.; Németh, A.; Zámbó, B.; Pákáski, M.; Janka, Z.; Sarkadi, B. Alterations of membrane protein expression in red blood cells of Alzheimer's disease patients. Alzheimer's Dement. Diagn. Assess. Dis. Monit. 2015, 1, 334–338.

69. Hegedus, T. Hegelab. Red Blood Cell Collection. 22 July 2015 Edition. Available online: (accessed on 9 February 2021).

70. Miyake, K.; Mickley, L.; Litman, T.; Zhan, Z.; Robey, R.; Cristensen, B.; Brangi, M.; Greenberger, L.; Dean, M.; Fojo, T.; et al. Molecular cloning of cDNAs which are highly overexpressed in mitoxantrone-resistant cells: Demonstration of homology to ABC transport genes. Cancer Res. 1999, 59, 8–13.

71. Özvegy, C.; Litman, T.; Szakács, G.; Nagy, Z.; Bates, S.; Váradi, A.; Sarkadi, B. Functional characterization of the human multidrug transporter, ABCG2, expressed in insect cells. Biochem. Biophys. Res. Commun. 2001, 285, 111–117.

72. Nakanishi, T.; Doyle, L.A.; Hassel, B.; Wei, Y.; Bauer, K.S.; Wu, S.; Pumplin, D.W.; Fang, H.-B.; Ross, U.D. Functional characterization of human breast cancer resistance protein (BCRP, ABCG2) expressed in the oocytes of Xenopus laevis. Mol. Pharmacol. 2003, 64, 1452–1462.

73. Homolya, L.; Orbán, T.I.; Csanády, L.; Sarkadi, B. Mitoxantrone is expelled by the ABCG2 multidrug transporter directly from the plasma membrane. Biochim. Biophys. Acta BBA Biomembr. 2011, 1808, 154–163.

74. Robey, R.W.; Medina-Pérez, W.Y.; Nishiyama, K.; Lahusen, T.; Miyake, K.; Litman, T.; Senderowicz, A.M.; Ross, D.D.; Bates, S.E. Overexpression of the ATP-binding cassette half-transporter, ABCG2 (Mxr/BCrp/ABCP1), in flavopiridol-resistant human breast cancer cells. Clin. Cancer Res. 2001, 7, 145–152.

75. Volk, E.L.; Schneider, E. Wild-type breast cancer resistance protein (BCRP/ABCG2) is a methotrexate polyglutamate transporter. Cancer Res. 2003, 63, 5538–5543.

76. Burger, H.; Van Tol, H.; Boersma, A.W.M.; Brok, M.; Wiemer, E.A.C.; Stoter, G.; Nooter, K. Imatinib mesylate (STI571) is a substrate for the breast cancer resistance protein (BCRP)/ABCG2 drug pump. Blood 2004, 104, 2940–2942.

77. Yang, C.-H.; Schneider, E.; Kuo, M.-L.; Volk, E.L.; Rocchi, E.; Chen, Y.-C. BCRP/MXR/ABCP expression in topotecan-resistant human breast carcinoma cells. Biochem. Pharmacol. 2000, 60, 831–837.

78. Elkind, N.B.; Apáti, A.; Várady, G.; Ujhelly, O.; Szabó, K.; Homolya, L.; Buday, L.; Német, K.; Sarkadi, B.; Szentpétery, Z.; et al. Multidrug transporter ABCG2 prevents tumor cell death induced by the epidermal growth factor receptor inhibitor iressa (ZD1839, gefitinib). Cancer Res. 2005, 65, 1770–1777.

79. Hegedüs, C.; Truta-Feles, K.; Antalffy, G.; Várady, G.; Német, K.; Özvegy-Laczka, C.; Kéri, G.; Örfi, L.; Szakacs, G.; Settleman, J.; et al. Interaction of the EGFR inhibitors gefitinib, vandetanib, pelitinib and neratinib with the ABCG2 multidrug transporter: Implications for the emergence and reversal of cancer drug resistance. Biochem. Pharmacol. 2012, 84, 260–267.

80. Li, J.; Cusatis, G.; Brahmer, J.; Sparreboom, A.; Robey, R.W.; Bates, S.E.; Hidalgo, M.; Baker, S.D. Association of variant ABCG2 and the pharmacokinetics of epidermal growth factor receptor tyrosine kinase inhibitors in cancer patients. Cancer Biol. Ther. 2007, 6, 432–438.

81. Ozvegy-Laczka, C.; Hegedűs, T.; Várady, G.; Ujhelly, O.; Schuetz, J.D.; Váradi, A.; Kéri, G.; Örfi, L.; Német, K.; Sarkadi, B.; et al. High-affinity interaction of tyrosine kinase inhibitors with the ABCG2 multidrug transporter. Mol. Pharmacol. 2004, 65, 1485–1495.

82. Telbisz, A.; Hegedüs, C.; Özvegy-Laczka, C.; Goda, K.; Várady, G.; Takáts, Z.; Szabó, E.; Sorrentino, B.P.; Váradi, A.; Sarkadi, B. Antibody binding shift assay for rapid screening of drug interactions with the human ABCG2 multidrug transporter. Eur. J. Pharm. Sci. 2012, 45, 101–109.

83. Shukla, S.; Robey, R.W.; Bates, S.E.; Ambudkar, S.V. Sunitinib (sutent, SU11248), a small-molecule receptor tyrosine kinase inhibitor, blocks function of the ATP-Binding Cassette (ABC) transporters p-glycoprotein (ABCB1) and ABCG2. Drug Metab. Dispos. 2008, 37, 359–365.

84. Ozvegy-Laczka, C.; Hegedus, C.; Szakács, G.; Sarkadi, B. Interaction of ABC multidrug transporters with anticancer protein kinase inhibitors: Substrates and/or inhibitors? Curr. Cancer Drug Targ. 2009, 9, 252–272.

85. Robey, R.W.; Honjo, Y.; Morisaki, K.; Nadjem, T.A.; Runge, S.; Risbood, M.; Poruchynsky, M.S.; Bates, S.E. Mutations at amino-acid 482 in the ABCG2 gene affect substrate and antagonist specificity. Br. J. Cancer 2003, 89, 1971–1978.

86. Damiani, D.; Tiribelli, M.; Calistri, E.; Geromin, A.; Chiarvesio, A.; Michelutti, A.; Cavallin, M.; Fanin, R. The prognostic value of P-glycoprotein (ABCB) and breast cancer resistance protein (ABCG2) in adults with de novo acute myeloid leukemia with normal karyotype. Haematology 2006, 91, 825–828.

87. Kim, J.E.; Singh, R.R.; Cho-Vega, J.H.; Drakos, E.; Davuluri, Y.; Khokhar, F.A.; Fayad, L.; Medeiros, L.J.; Vega, F. Sonic hedgehog signaling proteins and ATP-binding cassette G2 are aberrantly expressed in diffuse large B-Cell lymphoma. Mod. Pathol. 2009, 22, 1312–1320.

88. Sauerbrey, A.; Sell, W.; Steinbach, D.; Voigt, A.; Zintl, F. Expression of the BCRP gene (ABCG2/MXR/ABCP) in childhood acute lymphoblastic leukaemia. Br. J. Haematol. 2002, 118, 147–150.

89. Suvannasankha, A.; Minderman, H.; O'Loughlin, K.L.; Nakanishi, T.; Ford, L.A.; Greco, W.R.; Wetzler, M.; Ross, D.D.; Baer, M.R. Breast cancer resistance protein (BCRP/MXR/ABCG2) in adult acute lymphoblastic leukaemia: Frequent expression and possible correlation with shorter disease-free survival. Br. J. Haematol. 2004, 127, 392–398.

90. Plasschaert, S.L.A.; Van Der Kolk, D.M.; De Bont, E.S.J.M.; Vellenga, E.; Kamps, W.A.; De Vries, E.G.E. Breast Cancer Resistance Protein (BCRP) in acute leukaemia. Leuk. Lymphoma 2004, 45, 649–654.

91. Burger, H.; Foekens, J.A.; Look, M.P.; Gelder, M.E.M.-V.; Klijn, J.G.M.; Wiemer, E.A.C.; Stoter, G.; Nooter, K. RNA expression of breast cancer resistance protein, lung resistance-related protein, multidrug resistance-associated

proteins 1 and 2, and multidrug resistance gene 1 in breast cancer: Correlation with chemotherapeutic response. Clin. Cancer Res. 2003, 9, 827–836.

92. Faneyte, I.F.; Kristel, P.M.; Maliepaard, M.; Scheffer, G.L.; Scheper, R.J.; Schellens, J.H.; van de Vijver, M.J. Expression of the breast cancer resistance protein in breast cancer. Clin Cancer Res 2002, 8, 1068–1074.
93. Kanzaki, A.; Toi, M.; Nakayama, K.; Bando, H.; Mutoh, M.; Uchida, T.; Fukumoto, M.; Takebayashi, Y. Expression of multidrug resistance-related transporters in human breast carcinoma. Jpn. J. Cancer Res. 2001, 92, 452–458.