Minireview

Roles of the ABCG2 transporter in protoporphyrin IX distribution and toxicity

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Running Title: ABCG2 modulates PPIX distribution and toxicity

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Abstract:

ATP-binding cassette transporter subfamily G member 2 (ABCG2) is a membrane-bound transporter responsible for the efflux of various xenobiotics and endobiotics, including protoporphyrin IX (PPIX), an intermediate in the heme biosynthesis pathway. Certain genetic mutations and chemicals impair the conversion of PPIX to heme and/or increase PPIX production, leading to PPIX accumulation and toxicity. In mice, deficiency of ABCG2 protects against PPIX-mediated phototoxicity and hepatotoxicity by modulating PPIX distribution. In addition, in vitro studies revealed that ABCG2 inhibition increases the efficacy of PPIX-based photodynamic therapy by retaining PPIX inside target cells. In this review, we discuss the roles of ABCG2 in modulating the tissue distribution of PPIX, PPIX-mediated toxicity, and PPIX-based photodynamic therapy.

Significance statement

This review summarized the roles of ABCG2 in modulating PPIX distribution and highlighted the therapeutic potential of ABCG2 inhibitors for the management of PPIX-mediated toxicity.

Key Words:

ABCG2; transporter; protoporphyrin IX; distribution; toxicity; photodynamic therapy
Abbreviation list

ABCG2, ATP-binding cassette transporter subfamily G member 2; ALA, 5-aminolevulinic acid; ALAS, 5-aminolevulinic acid synthase; CAR, constitutive androstane receptor; EPP, erythropoietic protoporphyria; FECH, ferrochelatase; OATP, organic anion transporting polypeptides; PDT, photodynamic therapy; PPIX, protoporphyrin IX; PXR, pregnane X receptor; ROS, active oxygen species; SNP, single nucleotide polymorphism; XLP, X-linked protoporphyria
1. Introduction

ATP-binding cassette transporter subfamily G member 2 (ABCG2), alternatively known as breast cancer resistant protein, is an efflux transporter responsible for transporting various substances (Taylor et al., 2017). Many xenobiotics are ABCG2 substrates including certain dietary carcinogens, photosensitizers, and therapeutic drugs (van Herwaarden et al., 2003; Robey et al., 2005; Shukla et al., 2006; Nakanishi et al., 2013; Kukal et al., 2021). ABCG2 also transports endobiotics, such as protoporphyrin IX (PPIX), uric acid, melatonin, and calcifediol (Woodward et al., 2009; Taylor et al., 2017; Alvarez-Fernandez et al., 2023; Peng et al., 2023). The tissue distribution of ABCG2 includes but is not limited to the liver, erythrocytes, and kidney (Maliepaard et al., 2001; Zhou et al., 2005; Huls et al., 2008). Individual differences in ABCG2 are widely recognized as many single nucleotide polymorphisms (SNP) of ABCG2 have been identified in humans (Niall et al., 2018).

PPIX is an intermediate in the heme biosynthesis pathway and is a substrate of ABCG2 (Gibson et al., 1958; Jonker et al., 2002; Phillips, 2019; Wang et al., 2019). PPIX and heme are produced in the largest amounts in the bone marrow followed by the liver (Gibson et al., 1958; Sachar et al., 2016a; Phillips, 2019). The heme biosynthesis pathway begins with the reaction between glycine and succinyl-CoA to produce 5-aminolevulinic acid (ALA) (Figure 1), which is catalyzed by 5-aminolevulinic acid synthase (ALAS) (Gibson et al., 1958; Phillips, 2019). After six subsequent enzymic reactions, the final step of the heme biosynthesis pathway is the insertion of iron into PPIX to produce heme, which is catalyzed by ferrochelatase (FECH) (Gibson et al., 1958; Riethmueller and Tuppy, 1964; Sachar et al., 2016a; Phillips, 2019).
Dysfunctions of certain enzymes in the heme biosynthesis pathway can cause PPIX accumulation (Sachar et al., 2016a). Erythropoietic protoporphyria (EPP) is an autosomal recessive disease caused by FECH mutation (Rufenacht et al., 1998). Estimated prevalence varies in different areas ranging from 1/75,000 to 1/200,000 (Balwani, 2019). In EPP, loss-of-function mutations of FECH result in PPIX accumulation in erythroid cells, plasma, and the liver (Rufenacht et al., 1998; Whatley et al., 2008). A commonly used mouse model of EPP was developed from a base pair substitution in FECH (Fech-mut), resulting in a 2.7-6.3% normal FECH activity in homozygotes and 45-65% normal FECH activity in heterozygotes (Tutois et al., 1991; Boulechfar et al., 1993). Heterozygous Fech-mut mice show neither photosensitivity nor liver damage as in EPP while homozygous Fech-mut mice display clinical and biochemical features of severe EPP (Tutois et al., 1991; Yasuda and Desnick, 2019). Another EPP mouse model was generated from exon 10 deletion, which exhibited skin photosensitivity but no liver disease in heterozygotes (Magness et al., 2002).

Gain of function mutations of ALAS2 can cause PPIX accumulation, leading to X-linked protoporphyria (XLP) (Whatley et al., 2008). XLP is neither X-linked recessive nor X-linked dominant because heterozygous female displays variable phenotypes ranging from asymptomatic to normal symptom as an affected individual (Balwani and Desnick, 1993). Roughly 2-10% patients with EPP symptoms actually have XLP (Balwani, 2019). So far, no mouse model has been generated for XLP (Yasuda and Desnick, 2019). In addition to genetic factors, certain porphyrinogenic chemicals, such as N-methyl protoporphyrin IX, can cause PPIX accumulation by inhibiting FECH and/or inducing ALAS (Figure 1) (Cole and Marks, 1984; Hamilton et al., 1988; Fraser et al., 2003; Podvinec et al., 2004; Schauder et al., 2010; Gupta et al., 2013).
After sunlight exposure, PPIX can be excited because of its electron-rich aromatic porphyrin ring, leading to the formation of reactive oxygen species (ROS) and phototoxicity in EPP and XLP patients (Takeshita et al., 2004). Based on the same property of PPIX, PPIX-based photodynamic therapy (PDT) has been developed against cancer (Dolmans et al., 2003; Ishizuka et al., 2011). Accumulation of PPIX in EPP and XLP can also cause liver damage due to PPIX-mediated biliary blockage (Macdonald and Nicholson, 1976). ABCG2 is centrally involved in PPIX distribution and therefore can affect the severity of PPIX-mediated toxicity (Figure 1) (Jonker et al., 2002; Wang et al., 2019). This paper summarized our current understanding of the roles of ABCG2 in the tissue distribution and toxicity of PPIX. We accessed PubMed (https://pubmed.ncbi.nlm.nih.gov/) as our major source for this review.

2. Identification of PPIX as a substrate of ABCG2

In 2002, Jonker et al. generated Abcg2 knockout mice and explored the role of ABCG2 in pheophorbide a distribution and toxicity (Jonker et al., 2002). Since the structure of PPIX is similar to that of pheophorbide a, they also tested the PPIX level and found a significant increase of PPIX in the erythrocytes of Abcg2 knockout mice (Jonker et al., 2002), indicating that PPIX is a substrate of ABCG2. Later in 2005, Zhou et al. showed that erythroid cells with ABCG2 overexpression have a significant decrease of intracellular PPIX and this decrease can be reversed by ABCG2 inhibitors (Zhou et al., 2005). These results provided additional evidence for the role of ABCG2 in transporting PPIX out of erythroid cells (Zhou et al., 2005). Furthermore, Tamura et al. found that site-specific mutations of ABCG2 in insect cells caused defective PPIX
transport and photosensitivity (Tamura et al., 2006). All these findings support the idea that PPIX is a substrate of ABCG2.

3. Role of ABCG2 in the hepatic distribution of PPIX and PPIX-mediated hepatotoxicity

3.1. PPIX-mediated hepatotoxicity

Hepatic accumulation of PPIX and liver damage occur in both EPP and XLP (Rufenacht et al., 1998; Whatley et al., 2008). The liver, specifically the hepatobiliary system, is responsible for PPIX excretion from the body (Sachar et al., 2016a). Due to the hydrophobic nature of PPIX, high levels of PPIX in bile will precipitate and block bile ducts, impairing bile flow and resulting in damages to both hepatocytes and cholangiocytes (Perez-Barriocanal et al., 1989). Eventually, PPIX-mediated bile duct blockage can cause hepatic inflammation, fibrosis, and cirrhosis, mainly around portal areas (zone 1) (Anstey and Hift, 2007). Up to 20% of EPP and XLP patients suffer from liver injury and 1-5% of them develop severe complications including liver failure (Balwani et al., 2017; Balwani, 2019; Wensink et al., 2022). Liver injury in EPP/XLP typically begins in adulthood but several early onset cases are known (Meerman, 2000; Khalili et al., 2012). In addition to the genetic reasons that cause PPIX accumulation and hepatotoxicity, some chemicals, such as FECH inhibitors, ALAS inducers, and porphyrinogens, can also cause PPIX accumulation in the liver and result in liver damage in various degrees of severity (Cole and Marks, 1984; Fickert et al., 2007; Gupta et al., 2013; Li et al., 2013; Liu et al., 2015; Sachar et al., 2016b; Xu et al., 2022; Hussain et al., 2023).

3.2. Expression of ABCG2 in the liver
ABCG2 is broadly expressed in various types of liver cells, including hepatocytes and Kupffer cells (Maliepaard et al., 2001; Uhlen et al., 2015). ABCG2 is localized in the canalicular plasma membrane of hepatocytes (Figure 2), where it is responsible for the efflux of many xenobiotics and endobiotics from hepatocytes into the biliary system (van Herwaarden et al., 2003; Robey et al., 2005; Shukla et al., 2006; Blazquez et al., 2012; Nakanishi et al., 2013; Taylor et al., 2017; Kukal et al., 2021). The function of ABCG2 in Kupffer cells is poorly understood. The total amount of ABCG2 protein in the liver decreases slightly with age (Prasad et al., 2013; Riches et al., 2015). In addition, hepatic ABCG2 expression is significantly higher in male than female according to a study using both human liver samples and mice (Merino et al., 2005). Based upon a preclinical study using mice and rats, the gender difference of ABCG2 expression is thought to be associated with the inductive effect of testosterone (Tanaka et al., 2005). Because PPIX is a substrate of ABCG2, the expression of ABCG2 in the liver significantly affects the hepatic distribution of PPIX and PPIX-mediated hepatotoxicity (Jonker et al., 2002; Wang et al., 2019).

3.3. Role of ABCG2 in the distribution of PPIX to the liver in EPP and XLP

Under EPP and XLP condition, PPIX is mainly produced in bone marrow and enriched in erythroid cells (Gibson et al., 1958; Sachar et al., 2016a; Phillips, 2019). ABCG2 in erythroid cells is responsible for transporting PPIX out of cells into plasma (Figure 2) (Jonker et al., 2002; Zhou et al., 2005). Albumin is considered as the major endogenous carrier of PPIX in plasma while PPIX may also bind to other plasma proteins including immunoglobulin G and hemopexin (Seery and Muller-Eberhard, 1973; Cohen and Margalit, 1990; Brancaleon and Moseley, 2002; Sulkowski et al., 2016). Little is known for how protein binding of PPIX in plasma might affect PPIX disposition. Plasma PPIX is taken up by hepatocytes through unclear mechanisms.
Although hepatic organic anion transporting polypeptides (OATP) have been known to bind porphyrin substrates, no solid evidence is available to confirm that PPIX is a substrate of OATP (Shen et al., 2016). ABCG2 in hepatocytes transports PPIX into bile ducts, finally to the intestine and feces (Ibrahim and Watson, 1968; Sachar et al., 2016a), suggesting that ABCG2 plays an important role in controlling the disposition of PPIX (Figure 2).

3.4. Role of ABCG2 in PPIX-mediated hepatotoxicity in EPP and XLP

PPIX-mediated bile duct blockage is a critical step in the development of liver damage in EPP and XLP (Macdonald and Nicholson, 1976; Balwani et al., 2017; Balwani, 2019; Wensink et al., 2022). ABCG2 directly transports PPIX from hepatocytes into the biliary system (Figure 2). In Fech-mut mice, bile duct blockage and liver damage are evident, but these phenotypes were abolished in the Fech-mut mice with ABCG2 deficiency (Fech-mut/Abcg2-null) (Wang et al., 2019). In addition, deficiency of ABCG2 prevents PPIX-mediated bile duct blockage and hepatotoxicity caused by porphyrinogenic chemicals such as diethoxycarbonyl-1,4-dihydrocollidine and griseofulvin (Wang et al., 2019). These results are consistent with another preclinical study showing that retention of PPIX in mouse hepatocytes and Kupffer cells rather than in the biliary system alleviates PPIX-mediated hepatotoxicity (Lyoumi et al., 2011). These observations suggest that ABCG2 is a key mediator for PPIX-mediated hepatotoxicity and ABCG2 inhibition may be viable as a therapeutic approach against PPIX-mediated hepatotoxicity.

4. Role of ABCG2 in the dermal distribution of PPIX and PPIX-mediated phototoxicity

4.1. PPIX-mediated phototoxicity
PPIX accumulation in EPP and XLP can cause severe skin phototoxicity (Thapar and Bonkovsky, 2008; Balwani et al., 2017; Balwani, 2019). Upon sunlight exposure, a high circulation level of PPIX can be excited, resulting in ROS generation and skin damage (Balwani et al., 2017). Histological analysis revealed dermal necrosis and dermal endothelial cells seemed to be the primary target of PPIX-induced phototoxicity during acute sunlight exposure (Peterka et al., 1965; Brun and Sandberg, 1991; Lecha et al., 2009; Ahmed Jan and Masood, 2024). Chronically, deposition of hyaline materials in the blood vessel walls of dermal vascular plexuses are observed (Ahmed Jan and Masood, 2024). The most common symptoms in EPP include skin itching, pain, swelling, and erythema (Balwani, 2019). Blistering and scarring may occur with prolonged sunlight exposure (Thapar and Bonkovsky, 2008). In addition, leathery skin thickening may become evident on the knuckles, face, and other exposed surfaces (Thapar and Bonkovsky, 2008). The secondary effects of PPIX-mediated phototoxicity, including vitamin D deficiency due to light avoidance, have been noted in the clinic, which have a negative impact on quality of life of EPP and XLP patients (Spelt et al., 2010; Wahlin et al., 2011; Naik et al., 2019).

4.2. Role of ABCG2 in the distribution of PPIX to the skin in EPP and XLP

The endothelial cells in dermal blood vessels are the likely target of PPIX-mediated phototoxicity (Brun and Sandberg, 1991). The majority of PPIX in the skin comes from erythroid cells because the bone marrow is the major site for PPIX production in EPP and XLP (Gibson et al., 1958; Sachar et al., 2016a; Phillips, 2019). The accumulated PPIX in erythroid cells is released into plasma through ABCG2 (Jonker et al., 2002; Zhou et al., 2005; Puy et al., 2010), and then directly exposed to endothelial cells in the skin (Figure 3), suggesting that ABCG2 is a
key mediator in the dermal distribution of PPIX. In a study using EPP mouse models, higher levels of PPIX in erythrocytes and roughly 10-fold lower levels of PPIX in the skin were observed in Fech-mut/Abcg2-null mice when compared to Fech-mut mice (Wang et al., 2019), indicating that ABCG2 is critical in regulating the amount of PPIX distributed to the skin.

4.3. Role of ABCG2 in PPIX-mediated phototoxicity in EPP and XLP

The detailed mechanism of PPIX-mediated phototoxicity is not clear (Hussain et al., 2023). In line with the role of ABCG2 in the distribution of PPIX from the bone marrow and through the circulation to the skin (Figure 3), ABCG2 in erythrocytic cells is likely a determinant of PPIX-mediated phototoxicity in EPP and XLP. A preclinical study using EPP mouse models showed less PPIX exposure to the skin in Fech-mut/Abcg2-null mice when compared to Fech-mut mice, which ultimately alleviated PPIX-mediated phototoxicity (Wang et al., 2019), providing strong evidence that ABCG2 plays an essential role in PPIX-mediated phototoxicity. Future work is expected to assess the efficacy of ABCG2 inhibitors in preventing skin phototoxicity in EPP and XLP.

5. Role of ABCG2 in PPIX-based photodynamic therapy (PDT)

5.1. PPIX-based PDT for cancer treatment

PPIX-based PDT has been used as a part of treatments for various types of cancers, such as nasal squamous cell carcinoma, hidradenocarcinoma, and basal cell carcinoma (Liao et al., 2021; Cao et al., 2023; Lu et al., 2023). The procedure of PDT starts with the delivery of photosensitizers to the target tumor cells (Henderson and Dougherty, 1992). Then, the photosensitizer absorbs photons from light of a specific wavelength to enter the excited triplet state (Dolmans et al.,
The activated photosensitizers react with various cellular substrates and oxygen to generate ROS, inducing tumor cell damage (Dolmans et al., 2003). A general selectivity of PDT against cancer cells can be achieved by molecule-specific localization of the photosensitizer and the specific light delivery to the target tissue (Agostinis et al., 2011). PPIX is a well-known photosensitizer (Kennedy and Pottier, 1992). In PPIX-based PDT for cancer therapy (Figure 4), the PPIX precursor ALA is administered locally or systematically followed by targeted delivery of light (Kennedy and Pottier, 1992; Morton et al., 2015; Morton et al., 2020). It has been proposed that the specificity of PPIX-based PDT is achieved through the Warburg effect, whereby cancer cells have less active FECH than normal cells, resulting in higher PPIX concentrations in cancer cells (Pascale et al., 2020; Pignatelli et al., 2023). However, the underlying mechanisms for the specificity of PPIX-based PDT for cancer therapy are rather complicated and require more studies to clarify (Kiening and Lange, 2022).

5.2. Expression of ABCG2 in cancer cells and its impact on the efficacy of PPIX-based PDT

ABCG2 functions to export substances out of cancer cells (Khot et al., 2020). Overexpression of ABCG2 is frequently observed in various cancer cells and can account for multidrug resistance (Allikmets et al., 1998; Mo and Zhang, 2012). Similar to chemotherapy for cancer treatment, resistance is common in PDT (Mossakowska et al., 2022; Hui et al., 2023). Since PPIX is a substrate of ABCG2, a higher ABCG2 activity can potentially decrease the overall efficacy of PPIX-based PDT by transporting PPIX out of target cancer cells (Figure 4) (Khot et al., 2020; Yang et al., 2020). Conversely, ABCG2 inhibitors may boost the efficacy of PPIX-based PDT by increasing PPIX accumulation in cancer cells (Robey et al., 2005; Bebes et al., 2011; Nakayama et al., 2016; Müller et al., 2020). Ko143, an ABCG2 inhibitor, was found in multiple studies to
significantly increase the efficacy of PPIX-based PDT in cancer cells (Robey et al., 2005; Bebes et al., 2011; Müller et al., 2020). In addition, certain clinically used drugs such as lapatinib and gefitinib also increased the efficacy of PPIX-based PDT in cancer cells via ABCG2 inhibition (Ishikawa et al., 2015; Palasuberniam et al., 2021; Mansi et al., 2022). Further in vivo studies are needed to verify the effect of ABCG2 inhibition on the efficacy of PPIX-based PDT for cancer treatment.

6. ABCG2 inhibitors for modulating PPIX distribution and toxicity

Currently, options available for the management of PPIX-induced phototoxicity in EPP and XLP include antioxidants, skin hyperpigmentation, opaque clothing, phototherapy, and bone marrow transplant (Diffey and Farr, 1991; Heerfordt et al., 2023). Afamelanotide, an analog of α-melanocyte stimulating hormone, is the only FDA approved drug for EPP treatment in adult which requires subcutaneous implant (Wensink et al., 2021). Because of the essential role of ABCG2 in PPIX distribution and toxicity, ABCG2 inhibitors have recently caught a lot of research attention. Fumitremorgin C, a natural product isolated from fungus Aspergillus Fumigatus, is a potent ABCG2 inhibitor (Table 1) (Rabindran et al., 2000). Despite its great inhibitory activity on ABCG2, Fumitremorgin C is neurotoxic (Allen et al., 2002). Several rounds of structural modifications based on Fumitremorgin C gave birth to Ko143 (Table 1), an analog with high ABCG2 inhibitory efficacy and low neurotoxicity (Allen et al., 2002). However, Ko143 suffers greatly from its poor metabolic stability and low oral bioavailability, making it undesirable as a drug candidate (Li et al., 2016; Liu et al., 2017; Zechner et al., 2023). The poor metabolic stability of Ko143 is due to a labile ester group that can be easily hydrolyzed to give an inactive metabolite (Li et al., 2016; Liu et al., 2017; Zechner et al., 2023). Numerous Ko143
derivatives have been synthesized to improve its metabolic stability (Li et al., 2016; Zechner et al., 2023; Zhu et al., 2023). Other ABCG2 inhibitors with distinctive structures have also been developed (Table 1), including tariquidar derivatives, quinazoline derivatives, and indole derivatives (Krapf et al., 2019; Antoni et al., 2020; Antoni et al., 2021).

An increasing number of drugs and food components have also been identified as ABCG2 inhibitors (Table 1) (Mao and Unadkat, 2015). Several tyrosine kinase inhibitors, including gefitinib and imatinib mesylate, are potent ABCG2 inhibitors (Houghton et al., 2004; Nakamura et al., 2005). Gefitinib directly inhibits ABCG2, decreasing the efflux of ABCG2 substrate topotecan from cells (Nakamura et al., 2005). Similarly, imatinib mesylate reverses ABCG2-mediated drug resistance of ABCG2 substrates (Houghton et al., 2004). As a substrate of ABCG2, imatinib inhibits other ABCG2 substrates competitively (Burger et al., 2004). Furthermore, some anti-HIV drugs, such as lopinavir, nelfinavir and saquinavir, have also been identified as ABCG2 inhibitors (Weiss et al., 2007). Genistein, a member of the isoflavone family identified in food and dietary supplements, was found to be an ABCG2 inhibitor (Eldasher et al., 2013). Genistein inhibited the release of ABCG2 substrate mitoxantrone in ABCG2-overexpressing cells, but not in ABCG2-negative cells (Zhang et al., 2004). Other isoflavones such as daidzein, coumestrol, and biochanin A, also showed inhibitory effect on ABCG2 (An and Morris, 2010; Tamaki et al., 2010).

Although ABCG2 inhibitor-related drug-drug interactions have been extensively studied (Houghton et al., 2004; Zhang et al., 2004), the impact of ABCG2 inhibitors on PPIX disposition in pathological conditions like EPP and XLP has not been characterized. Given the wide
existence of ABCG2 inhibitors in drug and food components, their interference in PPIX homeostasis should be taken into consideration especially for EPP/XLP patients. Indeed, it would be interesting to determine whether some dietary ABCG2 inhibitors would be beneficial for alleviating the disease conditions in EPP and XLP. Further development of potent and selective ABCG2 inhibitors is also expected for EPP/XLP therapy and PPIX-based PDT.

7. Individual differences of ABCG2 and their impact on PPIX distribution and toxicity

Many factors may cause individual differences of ABCG2, such as gene polymorphisms, gene regulation, and/or exposure to ABCG2 inhibitors (Turner et al., 2006; Jie and Wen, 2010; Mao and Unadkat, 2015; Niall et al., 2018), which can potentially affect PPIX distribution and toxicity.

7.1. Single nucleotide polymorphism (SNP) of ABCG2 gene in humans

Many SNPs of ABCG2 have been identified (Niall et al., 2018). Q141K (421 C>A) variant is one of the most common ABCG2 SNP, which results in a decreased expression of ABCG2 (Imai et al., 2002; Ai et al., 2006). Impact of SNPs on ABCG2 function has been studied using porphyrin as a substrate and normalized to ABCG2 protein level (Ai et al., 2006). Compared to wild type, Q141K and several other mutations of ABCG2 decreased porphyrin efflux from cells (Ai et al., 2006), suggesting that SNPs of ABCG2 can affect porphyrin transport. However, further investigations are required to determine whether these SNPs can alter PPIX efflux and contribute to individual differences in EPP and XLP patients.

7.2. Regulation of ABCG2 expression
ABCG2 expression can be regulated at transcriptional and post-transcriptional levels (Turner et al., 2006; Xie et al., 2008; Yu-Zhuo et al., 2009; Jie and Wen, 2010). Constitutive androstane receptor (CAR) and pregnane X receptor (PXR) are two important nuclear receptors that regulate genes involved in metabolism and transport of endobiotics and xenobiotics (Jie and Wen, 2010). Ligand-dependent activation of CAR and PXR upregulates ABCG2 expression (Gorczyca and Aleksunes, 2020). Hepatic ABCG2 levels increased in mice and rats that were pretreated with CAR activators TCPOBOP and phenobarbital, respectively (Xueqian et al., 2010). Similarly, 2-acetylaminofluorene upregulated Abcg2 expression in wild type mice, but not in Pxr-null mice, indicating the involvement of PXR in regulating ABCG2 (Alexander et al., 2006). Many other transcription factors, including peroxisome proliferator-activated receptor and aryl hydrocarbon receptor, also regulate ABCG2 expression (Emilie et al., 2006; Hirai et al., 2007; Kah Poh et al., 2010). In addition, ABCG2 can be regulated post-transcriptionally and post-translationally. MicroRNA-328 downregulates ABCG2 protein level through microRNA-directed ABCG2 RNA cleavage (Yu-Zhuo et al., 2009). Phosphorylation of ABCG2 at threonine 362 plays a critical role in ABCG2 membrane localization, as mutated threonine 362 retained ABCG2 protein in cytosol instead of plasma membrane (Xie et al., 2008). Furthermore, demethylation in the promoter region of ABCG2 gene increases both ABCG2 mRNA and protein expression (Turner et al., 2006; Nakano et al., 2008), indicating the epigenetic regulation of ABCG2. However, the relationship between ABCG2 regulation and PPIX homeostasis has not been thoroughly characterized and future studies are needed to explore this area.

8. Summary and perspectives
Evidence reviewed here indicates that ABCG2 can have an important influence on PPIX-mediated hepatotoxicity, phototoxicity, and PPIX-based PDT. As an efflux transporter of PPIX, ABCG2 is important for the distribution of PPIX from the bone marrow to the liver and skin in EPP and XLP, as much as it is important for the PPIX-mediated adverse effects in these tissues. Besides ABCG2, future investigations are needed to determine the uptake transporter of PPIX, especially for hepatocytes. Preclinical studies have demonstrated that PPIX-mediated hepatotoxicity and phototoxicity are dependent on ABCG2, suggesting that ABCG2 inhibitors have a great potential for preventing PPIX toxicity in EPP and XLP. Preclinical studies have also suggested that ABCG2 inhibitors may enhance the efficacy of PPIX-based PDT for cancer treatment. Further studies on ABCG2 inhibitors are in progress for these treatment applications. It is of interest to note that the goal of ABCG2 inhibition to enhance PPIX-based PDT is to retain PPIX within cancer cells and increase phototoxic destruction of these cells, whereas the goal of ABCG2 inhibition in EPP and XLP would be to retain PPIX within erythrocytes to reduce cutaneous photosensitivity and within hepatocytes to prevent bile duct blockage and subsequent progression of liver damage.
Author Contributions

Wrote or contributed to the writing of the manuscript: Qi, Q., Gu, R., Zhu, J., Anderson, K.E., and Ma, X.

Conflicts of Interest

X.M. and J.Z. are inventors on a patent (WO2020236901) and hold equity in Portal Therapeutics, Inc. K.E.A. reports receiving consulting fees, advisory board fees and grants to the university from Alnylam Pharmaceuticals, Recordati Rare Diseases, Mitsubishi Tanabe Pharma America and Disc Medicine.

Data Availability Statement

This review article contains no datasets generated or analyzed during the current study.
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Footnotes

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Figure Legends

**Fig. 1. PPIX production and the general role of ABCG2 in PPIX distribution and toxicity.**

In the heme biosynthesis pathway active in bone marrow and the liver, glycine and succinyl-CoA are the precursors for heme and its intermediates including 5-aminolevulinic acid (ALA) and protoporphyrin IX (PPIX). Overactivated 5-aminolevulinic acid synthase (ALAS) or nonfunctional ferrochelatase (FECH) can cause PPIX accumulation. ABCG2 is an efflux transporter of PPIX and contributes to PPIX tissue distribution and toxicity. N, nucleus; (↑), activation; (X), suppression.

**Fig. 2. Role of ABCG2 in the distribution of PPIX to the liver and PPIX-mediated hepatotoxicity in EPP and XLP.** The two important sources of PPIX in hepatocytes are the uptaking of PPIX from plasma (the major source originally from bone marrow) and de novo synthesis. ABCG2 in red blood cells (RBC) transports PPIX into plasma. In addition, ABCG2 in hepatocytes transports PPIX into bile ducts for final excretion. High levels of PPIX in bile ducts can cause blockage and cholestatic liver injury.

**Fig. 3. Role of ABCG2 in the distribution of PPIX to the skin in EPP and XLP.** Bone marrow is the major site for PPIX production. ABCG2 in RBC transports PPIX into plasma, which is then exposed to dermal endothelial cells (EC), the target cells for PPIX-mediated phototoxicity.
Fig. 4. Impact of ABCG2 on the efficacy of PPIX-based photodynamic therapy (PDT).

During PPIX-based PDT, ALA, a precursor of PPIX, is administrated to increase PPIX production in cancer cells. Accumulated PPIX in cells are excited by light with specific wavelength, generating ROS to induce cell death. ABCG2 potentially decreases the efficacy of PDT by transporting PPIX out of the target cancer cells.
Table 1. A selection of ABCG2 inhibitors.

<table>
<thead>
<tr>
<th>Name</th>
<th>IC50 (µM)</th>
<th>Class</th>
<th>Reference</th>
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<tbody>
<tr>
<td>Fumitremorgin C</td>
<td>1.0</td>
<td>Natural product from <em>Aspergillus Fumigatus</em></td>
<td>(Allen et al., 2002)</td>
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<tr>
<td>Ko143</td>
<td>~0.01</td>
<td>Fumitremorgin C derivative</td>
<td>(Allen et al., 2002)</td>
</tr>
<tr>
<td>K2</td>
<td>0.13</td>
<td>Ko143 derivative</td>
<td>(Zhu et al., 2023)</td>
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<tr>
<td>Compound 20</td>
<td>0.72</td>
<td>Ko143 derivative</td>
<td>(Zechner et al., 2023)</td>
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<td>Tariquidar</td>
<td>6.2</td>
<td>P-glycoprotein inhibitor</td>
<td>(Antoni et al., 2020)</td>
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<td>UR-MB108</td>
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<td>Tariquidar derivative</td>
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<td>Tyrosine kinase inhibitor</td>
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<td>Compound 19</td>
<td>0.10</td>
<td>Gefitinib derivative</td>
<td>(Silbermann et al., 2020)</td>
</tr>
<tr>
<td>Compound 31</td>
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<td>Gefitinib derivative</td>
<td>(Krapf et al., 2019)</td>
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<tr>
<td>Imatinib</td>
<td>9.6</td>
<td>Tyrosine kinase inhibitor</td>
<td>(Burger et al., 2004; Houghton et al., 2004)</td>
</tr>
<tr>
<td>Lopinavir</td>
<td>7.7</td>
<td>Protease inhibitor</td>
<td>(Weiss et al., 2007)</td>
</tr>
<tr>
<td>Nelfinavir</td>
<td>13.5</td>
<td>Protease inhibitor</td>
<td>(Weiss et al., 2007)</td>
</tr>
<tr>
<td>Saquinavir</td>
<td>27.4</td>
<td>Protease inhibitor</td>
<td>(Weiss et al., 2007)</td>
</tr>
<tr>
<td>Compound</td>
<td>Activity</td>
<td>Type</td>
<td>Reference</td>
</tr>
<tr>
<td>-------------------</td>
<td>----------</td>
<td>-----------------------------</td>
<td>------------------------------------</td>
</tr>
<tr>
<td>Genistein</td>
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<td>Natural product</td>
<td>(Tamaki et al., 2010)</td>
</tr>
<tr>
<td>Biochanin A</td>
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<td>(Tamaki et al., 2010)</td>
</tr>
<tr>
<td>Daidzein</td>
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<td>(Tamaki et al., 2010)</td>
</tr>
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<td>Coumestrol</td>
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</tr>
<tr>
<td>Botryllamide G</td>
<td>6.9</td>
<td>Natural product</td>
<td>(Strope et al., 2020)</td>
</tr>
<tr>
<td>Compound 9c</td>
<td>0.05</td>
<td>Chromone derivative</td>
<td>(Roussel et al., 2020)</td>
</tr>
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Fig. 1

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ABCG2

PPIX

Blood vessel

Hepatocyte

Bile duct

blockage &

liver injury

Feces

Bone

marrow

(EPP &

XLP)

RBC

ABCG2

De novo

synthesis

ABCG2

PPIX

Bile duct

Fig. 2