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ABCB6, an ABC transporter impacting drug response and disease

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Abstract

Recent findings have discovered how insufficiency of ATP-binding cassette (ABC) transporter, ABCB6 can negatively impact human health. These advances were made possible by, first finding that ABCB6 deficiency was the genetic basis for some severe transfusion reactions and second, by determining that functionally impaired ABCB6 variants enhanced the severity of porphyria, i.e., diseases associated with defects in heme synthesis. ABCB6 is a broad-spectrum porphyrin transporter that is capable of both exporting and importing heme and its precursors across the plasma membrane, and outer mitochondrial membrane, respectively. Biochemical studies have demonstrated that while ABCB6 influences the antioxidant system by reducing the levels of reactive oxygen species, the exact mechanism is currently unknown, though effects on heme synthesis are likely. Furthermore, it is unknown what biochemical or cellular signals determine where ABCB6 localizes in the cell. This review highlights the major recent findings on ABCB6 and focuses on details of its structure, mechanism, transport, contributions to cellular stress and current clinical implications.

Keywords

heme; Transporter; ABCB6; toxicity; liver; porphyrin

Introduction

Heme has a vital role in biological processes such as cytochrome P450 mediated detoxification, oxygen transport, circadian rhythm, regulation of transcription and translation, apoptosis, and microRNA processing (1, 2). The majority of heme synthesis occurs in the liver and red blood cells (RBC). In RBCs, heme joins with globin to form hemoglobin, the molecule that binds oxygen, thus allowing RBCs to shuttle oxygen. Exquisite controls typically ensure intracellular homeostasis of heme: disruptions in heme synthesis occur, either by genetic disorders or secondary to drug or toxicant exposure. Further, novel genetic modifiers, that prevent or minimize the damage due to the formation of photoreactive intermediates (porphyrins, protoporphyrins), secondary to disruptions in heme synthesis are just beginning to be discovered.

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The synthesis of heme is a coordinated effort between the cytosol and mitochondria (Figure 1). Defects in the enzymes producing heme, in either the liver or the RBC, can result in porphyrias (an elevation in heme intermediates: the protoporphyrins), which are classified, in part, according to their site of overproduction. Protoporphyrins also act as photosensitizers by causing the release of singlet oxygen in a light-dependent manner. Porphyrins also produce cell toxicity in a light-independent manner. Interestingly the cytotoxicity of protoporphyrin IX (PPIX) might be affected by its intracellular localization, as confocal microscopic localization studies have found PPIX not just in the mitochondria (as expected), but also in nuclear membranes and the cytosol (3). Such compartmentalization suggests a transport process is operative for PPIX and possibly other intermediates in the heme biosynthetic pathway. Transporter proteins provide a mechanism of subcellular compartmentalization and create organelle-specific concentrations of molecules.

Transporters promote the movement of substrates across biological membranes. Active transport is catalyzed by one of three energy sources: electrochemical or osmotic gradients or the hydrolysis of ATP. ATP-binding cassette (ABC) transporters use ATP to facilitate the transmembrane, energy-dependent flux of an array of structurally diverse compounds used in many biological processes. ABC transporters have crucial roles in a variety of biological processes, such as heme biosynthesis, iron-sulfur cluster formation, antigen presentation, and insulin secretion. Heme is lipophilic and readily associates with membranes, which led to the early misconception that porphyrins, including heme, easily traversed biological membranes. However, heme and its tetrapyrrole intermediates contain anionic, negatively-charged carboxylate side chains that restrict transmembrane diffusion. Thus, their vectorial movement across the lipid bilayer requires a transporter. Notably, a reduction of cellular energy results in intracellular heme redistribution. This suggests that intracellular heme gradients are maintained actively.

The ABCB6 gene (also known as MTABC3 and P-glycoprotein related protein (PRP)) encodes an 842 amino acid protein that belongs to the B sub-family of ABC transporters and is a known energy-dependent transporter of porphyrins (Table 1) involved in heme biosynthesis (Figure 1) (4). ABCB6 was originally identified in 1997 while screening for novel ABC transporter genes associated with drug resistance in the liver (5). Since then, the biological impact of ABCB6 has expanded to include: resistance to toxic metals (6), protection against oxidative (7) and phenylhydrazine stress (8), potential roles in promoting tumor growth and proliferation (5, 9, 10), acquired chemotherapeutic resistance (11–15), facilitation of *de novo* porphyrin biosynthesis (4, 16), and encoding the blood group system, Langereis (*Lan*) (17). It is unknown how many of these phenotypes are directly related to the function of ABCB6, or if these observations are related to downstream perturbations. These recent findings suggest ABCB6 might be a potential therapeutic target.

This review surveys the current findings on the ABC transporter, ABCB6, and discusses its role in porphyrin homeostasis, oxidative stress, and pathology of various disease states.

ABC transporters: Structure and Transport Mechanism

Generally, ABC transporters share a canonical architecture consisting of two transmembrane domains (TMD) of low homology that are responsible for substrate binding and translocation, and two highly conserved cytosolic nucleotide binding domains (NBD) that participate in ATP binding and hydrolysis. The NBDs can be further divided into the RecA-like domain and a helical domain. The RecA-like domain contains two β -sheets, Walker A & B motifs, and the signature “ABC” motif. The domains of eukaryotic ABC transporters are organized into so-called “full” and “half” transporters. Full-transporters contain four domains (two TMDs and two NBDs in a single polypeptide) and half-transporters contain two domains (one TMD and one NBD). Functional half-transporters are obligate homo- or heterodimeric complexes. ABCB6 is a “half-transporter” that functions as a homodimer (4, 18). Hydrophobicity, sequence homology, and web-based programs for topology prediction of transmembrane proteins (i.e., TMPred, CCTOP, or HMMTOP) suggest ABCB6 contains 11 transmembrane helices (TM), with the NBD projecting into the cytoplasm (Figure 2) (4). However, it has been postulated that ABCB6 deviates from the canonical ABC architecture in that monomeric ABCB6 with its one NBD, and two TMDs (TMs 1–5 and TMs 6–11) harbors an additional TMD that has been termed TMD0 (TM 1–5) (discussed below).

TMD0s have been previously observed among other half-transporters (i.e. TAP1, TAP2) (19) within the ABC “B” subfamily and act as docking sites for adaptor proteins (20). Recently, TAP1 and 2 were resolved to 6.5 Å using cryo-electron microscopy (21). However, a resolvable density was not observed for their respective TMD0s, suggesting that TMD0 is either highly flexible or unstructured. The structure of another TMD0 containing transporter, SUR1 (sulfonylurea receptor 1, ABC “C” subfamily member), was recently determined at ~6 Å (22) and 5.6 Å (23) via cryo-electron microscopy. The SUR1 TMD0 contained a 5-helix bundle that is stabilized by its interaction with Kir6.2 (22, 23), an inwardly rectifying potassium channel. Given TMD0 of SUR1 is necessary for channel assembly with Kir6.2 and TMD0 of TAP1/2 binds adaptor proteins, this suggest that TMD0 acts as a docking site that is stabilized through protein-protein interactions. TMD0 is crucial for ABCB6 stability and function. ABCB6 undergoes glycan modification at a single conserved atypical NXC site (typical site is NXS/T) in the amino terminus (24), and the NXC site is also involved in an intramolecular disulfide bond. The cysteines, located in the amino-terminus, are dominant factors stabilizing ABCB6 with glycosylation acting as a modifier of stability within the context of disulfide bond formation (24). Disruption of the disulfide bond produces retention, and ultimately degradation, of ABCB6 in the endoplasmic reticulum (ER). Likewise, this disulfide bond is conserved in SUR1 (and other ABC “C” subfamily members, e.g., MRP1) and disruption leads to loss of function by ER retention (24). *One study has suggested that TMD0 is important to lysosomal targeting and, in the absence of TMD0, the ABCB6 core domain appears to localize mostly to the plasma membrane. Interestingly, TMD0 was dispensible for dimerization and ATP binding, however it was unclear if it impacted catalytic function (25). Thus TMD0 does not appear to play an essential role in folding and membrane insertion, but its effect on catalytic activity is unknown. This study failed to show that the core domain was capable of binding or transporting known substrates, thus the impact of TMD0 on the structural stability of the*

core domains of ABCB6 remains ambiguous. Collectively, these studies suggest that proper folding of TMD0 regulates protein maturation of ABCB6. It is unknown if ABCB6 docks with partner proteins through TMD0-mediated interactions.

ABC transporter-mediated transport requires ATP hydrolysis. While the exact mechanism is unknown, available biochemical studies have led to several proposed models (Figure 3). The initial “Sequential Binding” model focused on ATP binding to one NBD followed by ATP hydrolysis at the other NBD. (sequential ATP binding and hydrolysis drove substrate transportation (26)). This was followed by the “ATP-switch” model, where the binding of ATP, not hydrolysis, drives translocation (27). Currently, the “ATP-switch” model has been further refined into variations of the “Alternating Access” model. The “Alternating Access” model proposes that substrates bind the ATP-less transporter which exists in a state open to the cytoplasm with high-affinity for substrate. Next, the binding of ATP promotes the power stroke for transport and the protein transitions to an occluded state bound to ATP and substrate. After ATP binds, the transition to the high-energy, post-hydrolysis intermediate switches TMD accessibility from inward-open to outward-open, where affinity for substrate is reduced leading to substrate expulsion (28, 29). However, it is not that simple as recent studies for P-glycoprotein (ABCB1) revealed a modified Alternating Access mechanism reminiscent of the Sequential Binding model with ATP hydrolysis driving the opening and closing of the TMDs to translocate substrate (30). Given the functional and substrate diversity of ABC transporters, it is likely that individual subtypes of ABC transporters have modified their underlying transport mechanism to optimize transport and substrate specificity in their niche.

Cellular Localization

Human ABCB6 was initially cloned and named MTABC3. It was localized to chromosome 2q35.5. Confocal microscopy and subcellular fractionation revealed that it resided in the mitochondria (31). Subsequent studies refined this subcellular location to the mitochondrial outer membrane (4, 32), despite the lack of an identifiable mitochondrial targeting sequence. Interestingly, subcellular fractionation studies by Paterson et. al. indicated that, depending upon cell type, ABCB6 localized to two predominant cellular locations, one mitochondrial and the other the plasma membrane (32). However, it was unknown if ABCB6 functioned as a porphyrin transporter at the plasma membrane. As mature RBCs lack mitochondria, ABCB6 was definitively localized to the red blood cell plasma membrane by Helias et. al. which was an important advance as it led to a molecular understanding of a rare recessively inherited blood group, *Lan* (17). Additional reports place it in the Golgi (33), ER (24) and the endolysosomal system (34). However, it is unknown if ABCB6 has any function in these latter locations or if they are either transit stops enroute to a final destination or simply variation in localization due to a robust overexpression system or use of a “tagged” ABCB6 of indeterminate function.

ABCB6 undoubtedly trafficks through the ER, as it exists as an *N*-glycosylated protein (2,17, 20). But as previously mentioned, ABCB6 has only a single atypical site of glycosylation, NXC, in its amino terminus (24). The cysteine in the NXC site is crucial to disulfide bond formation with C26 in the amino terminus and an inability to form this

disulfide bond resulted in ER accumulation and subsequent degradation of ABCB6 by the proteasome (24). Notably, the oxidizing environment of the ER lumen favors formation of the disulfide bond, unlike in the cytosol. ABCB6 likely reaches the plasma membrane via the trans-Golgi network, as blocking transit from the ER to the Golgi with Brefeldin A prevented the formation of ABCB6 with the mature Endo-H resistant glycans (24). The state of glycosylation or the oxidation state of the disulfide bond might affect ABCB6 trafficking. Notably, subcellular fractionation reveals the lower molecular weight form of ABCB6 in the mitochondrial fraction versus the higher molecular weight form that is found in the plasma membrane fraction (32). It is unknown if the mitochondrial and plasma membrane forms are differentially glycosylated. TMD0 has also been implicated in directing ABCB6 to endo-lysosomal vesicles (25). Currently, it is unknown if, in all tissues, ABCB6 traffics similarly or if metabolic conditions, such as porphyrin synthesis, direct ABCB6 to a specific subcellular location.

Porphyrin Transport, Cellular Stress, and Drug Transport Potential

The aforementioned inability of porphyrins to readily traverse membranes, their varying subcellular localization and redistribution by a change in cellular energy status suggested a transporter or transporters contributed to the intracellular porphyrin partitioning and movement. ABCB6 was found to be a mitochondrial heme binding protein that was capable of interacting with a variety of tetrapyrroles such as: heme, coproporphyrin III, PPIX, and a plant porphyrin, pheophorbide A (4). The inability of embryonic stem cells, lacking one allele of *Abcb6*, to fully engage in porphyrin biosynthesis suggested its absence might be lethal. Surprisingly, when *Abcb6*-null mice were generated, they appeared phenotypically normal (8). An evaluation of their hematological parameters revealed elevated erythroid PPIX levels with modest alterations in the mean corpuscular volume and hematocrit (8), findings suggesting a mild anemia that was also consistent with elevated Zn-PPIX, a byproduct formed when Fe^{+2} is not readily available for heme synthesis. Interestingly, *Abcb6*-null mice were noted for their compensatory increases in genes that positively influence porphyrin biosynthesis and iron homeostasis (i.e., FECH and EKLf) (8). Like other normal appearing ABC transporter knockout animals (e.g., ABCB1) (35), their inherent defects were not revealed until challenged. Likewise *Abcb6*-null mice displayed markedly defective erythropoiesis when stressed with phenylhydrazine, which, by destroying all mature RBCs, forces *de novo* RBC replacement (8). The elevated levels of protoporphyrin IX (PPIX) in *Abcb6*-null RBCs (8), suggest these animals might have more damaged RBCs, which could be due to free radical generation by excessive PPIX levels.

ABCB6 has also been shown to protect cells from oxidative stress (6). Due to ABCB6 ability to promote increased intracellular heme concentrations (7), a possible protective mechanism is to increase the amount of catalase (an enzyme that converts toxic hydrogen peroxide to water) by providing more heme, a co-factor that is critical to catalase stability and activity. Interestingly, ABCB6 overexpression reduced the amount of cytosolic reactive oxygen species (ROS) (4) and this protected against arsenite, the oxyanion of trivalent arsenic, which increases ROS by releasing reactive iron from ferritin (36). As chronic arsenite exposure affects mitochondrial electron transport complexes, it is conceivable that arsenite also impacts mitochondrial ROS. ABCB6 has also been implicated in mitigating

toxicity to copper (37), which increases ROS by interfering with the respiratory complexes I, II, and IV of the mitochondrial electron transport chain (38). Thus, while ABCB6 appears to modulate cytosolic ROS, secondary to providing heme for catalase, it is unknown if ABCB6 provides protection against ROS in the mitochondria. Further, it is unknown if non-functional or functionally impaired human variants of ABCB6 exacerbate conditions associated with mitochondrial dysfunction that are not directly related to porphyrin biosynthesis.

A number of studies have associated increased ABCB6 expression, with resistance to multiple chemotherapeutics such as the camptothecin, CPT-11 in A549 lung cancer cells (11), the combination of paclitaxel/FEC (5-fluorouracil, epirubicin, and cyclophosphamide) in breast cancer (12), daunorubicin in acute myeloid leukemia (39), and resistance to 5-fluorouracil, SN-38, and vincristine in KAS cells (40). ABCB6 up-regulation has also been linked to resistance of ovarian cancer cells to cisplatin, doxorubicin, methotrexate, paclitaxel, topotecan, and vincristine (41). However, these findings are difficult to reconcile with the mitochondrial heme/porphyrin importing function of ABCB6. With the recent demonstration that the plasma membrane ABCB6 is capable of exporting porphyrins, it is conceivable that ABCB6 exports chemotherapeutic agents too, but this has not been directly demonstrated. An alternative explanation for the association between ABCB6 and resistance to these diverse therapeutic compounds is related to their effect on ROS generation. Reportedly these chemotherapeutics, with the exception of SN-38, have been shown to induce ROS production (42–44). We propose that mitochondrial ABCB6 affects ROS mostly by regulating porphyrin biosynthesis, which limits the ability of chemotherapeutics to induce mitochondrial ROS formation and leads to alternative mechanisms of drug resistance. A future challenge will be to uncouple the mitochondrial function of ABCB6 from its role at the plasma membrane. One speculation is that ABCB6 exports molecules that facilitate the generation of intracellular ROS. However, the precise mechanisms and their potential impact remain to be determined.

Clinical Significance of ABCB6

In 1962, van der Hart and colleagues identified an antibody to a common RBC antigen that was related to a severe and immediate transfusion reaction. It was discovered because RBCs from this patient (Mr. Langereis and his brother), who experienced the transfusion reaction, were non-reactive to the antibody. Further examples of the so called, anti-Lan phenotype have been described with the clinical outcome being hemolytic transfusion reactions (ranging from none to severe) and hemolytic disease of the fetus and newborn, again with a range of severity. In 2012, Helias and colleagues, after developing a monoclonal antibody (anti-Lan, referred to as OSK43) (17) established the molecular identity of a new blood group, (*Lan*), which was due to the absence of ABCB6 expression on RBC membranes. ABCB6 mutations corresponding to the *Lan* phenotype can be found in Table 2. They further showed the *Lan*-null individuals had slightly higher RBC porphyrin levels, suggesting ABCB6 exported porphyrins. However, apart from elevated porphyrins, these individuals appeared normal and exhibited no obvious pathology.

The inherited porphyrias are metabolic disorders of porphyrin/heme biosynthesis caused by mutations in genes encoding enzymes in the heme biosynthetic pathway. The intermediates of heme biosynthesis are toxic (especially PPIX) and their buildup is particularly damaging to the liver, hematopoietic system, skin and neural tissues. Most porphyrias are inherited as autosomal dominant mutations with marked variability in phenotypes that cannot be accounted for by the porphyria mutation. Indeed, a long-standing clinical mystery has been why some porphyria patients become more ill than others, even when harboring the same heme biosynthetic defect. Recently, using a well-characterized cohort of porphyria patients coupled with an unbiased genomic analysis, it was determined by whole exome sequencing and pathway analysis that ABCB6 was a genetic modifier of porphyria (16), and corresponding rare variants are listed in Table 2. Plasma membrane ABCB6 was shown to be a high affinity exporter of cytotoxic porphyrins. Furthermore, it was established that the ABCB6 variants behaved like dominant negatives (16), and are likely degraded by the proteasome (Figure 4). From these findings a new porphyria-prone animal model was developed. Genetic deficiency of ABCB6 coupled with a well-characterized porphyria model (Fech deficiency (45)) produced greater porphyrin elevation in red blood cells, hepatocytes, excreta, and increased liver damage, some mice formed extensive adenomas (Figure 5) which associated with increased liver weights. Because mice heterozygous for *Abcb6* exhibited an intermediate porphyrin accumulation and excretion defect, it suggests that simply reducing ABCB6 function could produce porphyria among susceptible individuals. Further, these findings raise the possibility that drugs with the propensity to cause porphyria (see <http://www.porphyrifoundation.com/drug-database>) could exacerbate or produce severe life-threatening acute or chronic liver disease if they also inhibited ABCB6. This might be especially likely in individuals with ABCB6 insufficiency or deficiency. To our knowledge, this has not been assessed among individuals of the *Lan* blood group.

ABCB6 is highly expressed in megakaryocytes and increased platelet production occurs in mice deficient in ABCB6 (38). Because absence of ABCB6 enhances megakaryopoiesis, it was speculated that ABCB6 modulated the survival of megakaryocyte progenitors. This is probable because megakaryocytes progenitors highly express the porphyrin biosynthetic genes, and ABCB6 absence is likely to increase oxidative stress. Furthermore, *Abcb6*-null platelets are hyperactive, and when superimposed on a model of atherosclerosis, accelerated the development of atherosclerosis (46). This is consistent with the view that more active platelets are associated with more severe atherosclerotic lesions. These findings suggest elevating ABCB6 function might protect against atherosclerosis mediated by platelet activity.

ABCB6 has been identified as the causative gene for familial pseudohyperkalemia (Table 2), a mild, non-hemolytic subtype of hereditary stomatocytosis, a wide spectrum of inherited hemolytic disorders in which the basal RBC membranes have enhanced cation permeability. Familial pseudohyperkalemia is a dominant RBC trait, causing a temperature-dependent anomaly in the plasma membrane that produces RBCs that are “leaky” for potassium. The increased potassium permeability elevates potassium levels in whole blood stored at or below room temperature. Currently, this phenotype does not appear to be functionally linked to ABCB6 and porphyrin synthesis. However, we speculate that elevated levels of

porphyrins, due to the absence of functional ABCB6 (it is unknown if ABCB6 is nonfunctional in these individuals), might alter RBC potassium channels. Indeed, by analogy the large conductance calcium channel (SloBK) is known to be regulated by heme. Thus, dysregulation in heme and porphyrins in RBC may disrupt potassium channels, which is conceivable considering SUR2A (ABCC9), the regulator of the K_{ATP} potassium channel, has been recently shown to be regulated by heme binding (47).

ABCB6 has also been implicated in other genetic diseases such as to ocular coloboma (a missing piece of tissue in the eye) and dyschromatosis universalis, which is a genetic disorder of abnormal pigmentation (48, 49). Reported variants associated with these disorders are found in Table 2. However, to date, these phenotypes have not been directly linked to ABCB6 function as a porphyrin transporter. Alternatively, it is possible these diseases are only genetic (and not functional) associations with the ABCB6 locus (2q35.5).

There have been reported correlations between increased ABCB6 mRNA levels in some cancers, such as intra-hepatic recurrence of hepatitis C virus-related hepatocellular carcinoma (50), prostate cancer (51), and melanoma (13). One could speculate that increased expression of ABCB6 is related to an increased heme biosynthesis in tumors (4, 7, 8, 52). An additional possibility is that ABCB6 is transcriptionally upregulated in cancers. ABCB6 possesses, in its promoter a >1000bp CpG island (5); hepatoma cell lines treated with the demethylating agent, 5-aza-2'-deoxycytidine, displayed up to a 4-fold elevation in ABCB6 mRNA level, a finding consistent with activation of ABCB6 transcription. Thus, given the propensity for an alteration in DNA methyl-transferases in cancer, it is possible ABCB6 is upregulated during carcinogenesis. However, it is also conceivable that the cancer-associated increases in ABCB6 expression are because it provides a survival advantage to the tumor. This seems likely as patients with hepatitis C virus-related hepatocellular carcinoma and high ABCB6 mRNA display much worse overall survival (45). Interestingly, ABCB6 expression in gliomas is also strongly correlated with histological grade (53). It remains to be determined if ABCB6, beyond its effects on heme/porphyrin synthesis, alters the metabolic phenotype of tumors or if ABCB6 modifies disease progression.

Perspective

The biomedical importance of ABCB6 is that it contributes to cellular porphyrin homeostasis. But there are a number of unanswered questions. A pivotal unanswered question is if drugs and xenotoxins that cause porphyria do so by affecting ABCB6 function. Further because the synthesis of heme from porphyrinogen precursors is coordinated with iron delivery to the mitochondria, it is unknown if ABCB6 absence affects this process. However, given, the rare porphyrin, zinc-protoporphyrin IX is formed when heme synthesis is increased in the absence of ABCB6 (8), one suspects this might be the case. Perturbation or alteration in iron-delivery to intracellular sites, coupled with altered heme synthesis, might lead to an increase in toxic ROS.

We do not know if ABCB6 dysfunction produces an alteration in iron homeostasis. Ferroptosis involves iron-dependent accumulation of lipid associated ROS (51) and is an oxidative, iron-dependent form of cell death that is distinct from apoptosis, classic necrosis,

autophagy and other forms of cell death. If ABCB6 does produce alterations in the intracellular balance of iron, then ferroptosis might also account for the increased disease severity (not just the porphyrins) in porphyric patients. It has not been determined if ABCB6 contributes to ferroptosis, because how it contributes to iron homeostasis has not been elucidated. Clearly future studies will need to determine how ABCB6 impacts iron homeostasis. If ABCB6 role in intracellular iron homeostasis is established, we speculate that inhibitors of ABCB6 could be used to therapeutic advantage as a chemotherapeutic strategy to activate ferroptosis.

An important unanswered question is why ABCB6 trafficks to the mitochondria and plasma membrane. A simple answer might be that it fulfills roles in heme/porphyrin homeostasis. However, it has not been directly demonstrated that ABCB6 changes location with the state of heme biosynthesis. Moreover, what are the signals that contribute to this? Does glycosylation contribute to trafficking? Or do mutations in TMD0 impact membrane targeting? Zebrafish ABCB6 lacks the consensus site for glycosylation and therefore would make an excellent model for understanding if glycosylation plays a regulatory role in localization. Given, TMD0 in other ABC transporters has been shown to mediate protein-protein interactions, we speculate that TMD0 mediates interactions with chaperones or other effector proteins that aid in assembling tertiary structural elements or guiding ABCB6 to its final destination. This is exemplified in the *Lan* mutant R192Q (in TMD0) where near normal ATP and heme binding is observed, but surface expression is severely diminished (16) or absent (17). This suggests TMD0 has an important, but currently undetermined role that is independent of substrate binding and translocation. While a wealth of knowledge has been discovered in the last 10 years, the contribution of ABCB6 to physiology and the pathology of various disease states remain in its infancy. Future studies will undoubtedly elucidate more detailed structure-based functional models and determine the range of substrates (both endogenous and exogenous) that ABCB6 transports.

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Figure 1.

ABCB6 is an integral membrane transporter in the heme biosynthetic pathway. This figure depicts the role of ABCB6 in heme and porphyrin biosynthesis, other avenues of porphyrin uptake have been excluded. We speculate that when ABCB6 function is altered metabolites proceed through a nonenzymatic branch of the pathway resulting in accumulation of uroporphyrinogen I and coproporphyrinogen I. The first step in the heme biosynthetic pathway, the condensation of glycine and succinyl CoA by ALA synthase, has been omitted for clarity. Note: A non-energy dependent route for mitochondrial porphyrin uptake has been noted (see reference #8), but its properties have not been defined.

Figure 2.

Predicted membrane topology of monomeric ABCB6. ABCB6 has been shown to have the amino terminus in the intermembrane space of the mitochondria. Given, that ABCB6 exports CPIII when localized to the plasma membrane, the amino terminus is likely in the cytoplasm when ABCB6 is localized to the plasma membrane, as ABCB6 has not been shown to facilitate bidirectional transport. Likewise, the nucleotide binding domain (NBD) is orientated opposite of the amino terminus. Confirmed sites of glycosylation are shown in magenta, disulfide bonds in blue, conserved NBD motifs are in red (in sequential order: Walker A, Q-loop, Signature, Walker B, D-loop, and H-loop), and residues within the NBD are shown in yellow.

Figure 3.

Evolution of the molecular transport mechanisms of ABC “B” transporters. Sequential binding model: The catalytic site A is empty, the B site has bound ATP and substrate is bound in the inward-facing conformation. ATP binding at site B allows hydrolysis at site A, and hydrolysis is prohibited at the A site. The A site then relaxes allowing a conformational switch from inward-open to outward-open followed by the release of inorganic phosphate (P_i), ADP and substrate. A and B sites have reversed their relationship and are now ready to accept a new substrate (26). ATP-switch model: Substrate binds to inward-open conformation. Two molecules of ATP bind cooperatively. A conformational switch occurs from inward-facing to outward-facing and substrate is released. ATP is sequentially hydrolyzed. This is followed by the sequential release of P_i and ADP, restoring the transporter to the basal configuration (27). Alternating access model: Like the ATP-switch model, substrate binds to inward-open conformation. Then two molecules of ATP bind cooperatively. However, the protein structure transitions to an intermediate occluded state, sequestering the substrate and both ATPs. Sequential ATP hydrolysis initiates substrate release. The sequential release of P_i and ADP, restores the transporter to the inward-open configuration (28, 29). Energy transduction model: Substrate binds to the ATP loaded transporter. One ATP is occluded in the catalytic site, and is followed by a doubly occluded state, where the hydrolysis of one ATP and release of P_i . The transporter transitions to the outward-facing conformation after hydrolysis of the final ATP thereby releasing substrate and P_i . ADPs then dissociate, and the transporter returns to an inward-open configuration ready to bind two new molecules of ATP (30). Magneta, TMD1 ; Blue, TMD2 ; Purple,

NBD1; Green, NBD2; Brown box, substrate; Yellow circle, ATP; Red circle, ADP + P_i;
Orange circle, ADP.

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Figure 4. Mutant ABCB6 is degraded by the proteasome. MG132, a proteasome inhibitor, stabilizes known dominant negative mutation A492T.

Figure 5.

Loss of ABCB6 increases liver damage in mouse model of porphyria. Larger adenomas are observed for mice lacking ABCB6 and ferrochelatase (A). This is also associated with an increase in liver weight (B).

Table 1

Substrates of ABCB6

Substrate	Determined by	Reference
Hemin	Competitive pull down	(4)
Protoporphyrin IX	Competitive pull down	(4)
Coproporphyrin III	Competitive pull down, mitochondrial import, membrane vesicle transport	(4, 8, 16)
Coproporphyrin I	membrane vesicle transport	(16)
Uroporphyrin III	membrane vesicle transport	(16)
Uroporphyrin I	membrane vesicle transport	(16)
Pheophorbide A	Competitive pull down	(4)

Table 2

Nonsynonymous ABCB6 mutations associated with phenotypical alterations

Nucleic Acid*	Amino Acid	Phenotype	Outcome	Allele Frequency MAF % (EA/AA/all)**
574C>T	R192W	Lan	Loss of expression on RBC	0.2442/0.0/0.1615
575G>A	R192Q	Lan	Loss of expression on RBC	0.4186/0.0681/0.2999
826C>T	R276W	Lan	Loss of expression on RBC	1.3265/0.386/1.0078
1028G>A	R343Q	Lan	Loss of expression on RBC	0.1164/14.2271/4.8852
1762G>A	G588S	Lan	Loss of expression on RBC	0.6628/0.908/0.469
2216G>A	R739H	Lan	Loss of expression on RBC	NA
2215C>T	R739H	Lan	Loss of expression on RBC	0.0465/0.0/0.0308
197_198insG	A66fsX	Lan	Loss of expression on RBC	NA
717G>A	Q239X	Lan	Loss of expression on RBC	NA
953_956del	G318fsX	Lan	Loss of expression on RBC	NA
1533_1543dup	L515fsX	Lan	Loss of expression on RBC	NA
1709_1710del	E570fx	Lan	Loss of expression on RBC	0.0/0.0234/0.008
1690_1691del	M564fsX	Lan	Loss of expression on RBC	NA
1867_del-ins	G623fsX	Lan	Loss of expression on RBC	NA
1985_198del	L662fsX	Lan	Loss of expression on RBC	0.0121/0.0/0.008
1588_1559 ins	V520fsX	Lan	Loss of expression on RBC	NA
376del	V1269fsX	Lan	Loss of expression on RBC	NA
1942C>T	R648X	Lan	Loss of expression on RBC	0.0116/0.0/0.007
1236G>A	W412X	Lan	Loss of expression on RBC	NA
85_87del	F29del	Lan	Loss of expression on RBC	NA
1067T>C	L356P	Dyschromatosis universalis hereditaria	Undetermined	NA
508A>G	S170G	Dyschromatosis universalis hereditaria	Undetermined	NA
1736G>A	G579E	Dyschromatosis universalis hereditaria	Undetermined	NA
1358C>T	A453V	Dyschromatosis universalis hereditaria	Undetermined	0.0116/0.0/0.0077
964A>C	S322K	Dyschromatosis universalis hereditaria	Undetermined	NA
2431C>G	L811V	Ocular coloboma	Undetermined	NA
169G>A	A57T	Ocular coloboma	Undetermined	NA
1124G>A	R375Q	Familial pseudohyperkalemia	Undetermined, normal RBC expression	NA
1123C>T	R375W	Familial pseudohyperkalemia	Undetermined, normal RBC expression	NA

* This table was curated from the following references: (16, 17, 48, 49, 54–59)

** Allele Frequencies MAF% were obtained from NHLBI GO Exome Sequencing Project (ESP) Exome Variant Variant Server (<http://evs.gs.washington.edu/EVS/>) accessed on August 14, 2017.