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# Structure and Mechanism of Human ABC Transporters

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## Keywords

ATP-binding cassette transporter, membrane protein, human disease, drug extrusion, lipid homeostasis, cryo-EM

## Abstract

ABC transporters are essential for cellular physiology. Humans have 48 ABC genes organized into seven distinct families. Of these genes, 44 (in five distinct families) encode for membrane transporters, of which several are involved in drug resistance and disease pathways resulting from transporter dysfunction. Over the last decade, advances in structural biology have vastly expanded our mechanistic understanding of human ABC transporter function, revealing details of their molecular arrangement, regulation, and interactions, facilitated in large part by advances in cryo-EM that have rendered hitherto inaccessible targets amenable to high-resolution structural analysis. As a result, experimentally determined structures of multiple members of each of the five families of ABC transporters in humans are now available. Here we review this recent progress, highlighting the physiological relevance of human ABC transporters and mechanistic insights gleaned from their direct structure determination. We also discuss the impact and limitations of model systems and structure prediction methods in understanding human ABC transporters and discuss current challenges and future research directions.

## Contents





## INTRODUCTION

**Half-transporter:** an ABC transporter assembled as homodimers or heterodimers from two distinct halves, each bearing a transmembrane domain and a nucleotide binding domain, separately expressed

**Full transporter:** an ABC transporter assembled as a single polypeptide of two halves, each containing, at minimum, a transmembrane domain and a nucleotide binding domain

**Nucleotide binding domain (NBD):** the core domain of each ABC transporter half comprising structural elements responsible for nucleotide binding and hydrolysis

Members of the adenosine triphosphate binding cassette (ABC) transporter superfamily catalyze substrate translocation across cellular membranes and are ubiquitously expressed in all domains of life (33, 34, 59, 97). Humans have 48 ABC transporter genes encoding 44 membrane transporters that belong to five distinct families (A, B, C, D, and G) and display a wide array of substrate specificities and functionalities (16, 34, 59). Active in nearly all cells and tissues (**Figure 1**), these transporters play vital physiological roles ranging from lipid homeostasis to transport of diverse endogenous and exogenous compounds (**Table 1**). Their substrates include hormones, vitamins, lipids, sterols, fatty acids, peptides, and xenobiotic compounds, among others. Unsurprisingly, human ABC transporters hold tremendous biomedical and pharmacological relevance (16, 34, 59, 89). Several devastating pathologies result directly from dysfunction of these transporters, making them important targets for therapeutic intervention (60, 154). Structural biology has played a key role in advancing our understanding of ABC transporter architecture and has provided a general mechanistic framework for rationalizing their function. ABC transporters are organized as two symmetric halves that are expressed either (*a*) as separate subunits (half-transporters) that assemble as homodimers or heterodimers or (*b*) as monomers containing two nonidentical halves within a single polypeptide (full transporter). Each half comprises, at minimum, a nucleotide binding domain (NBD) that is responsible for ATP binding and hydrolysis and a transmembrane domain (TMD) that facilitates substrate export (away from the NBDs) or import (toward the NBDs) (**Figure 2*a***). Despite this shared basic architecture, ABC transporters display tremendous variability in substrate specificity, transport mechanisms, and regulation.

While experimentally determined structures of bacterial and archaeal homologs have been invaluable as model systems (5, 19, 29, 66, 98, 99, 119, 167), human ABC transporters have proven more averse to direct characterization, and their high-resolution structures have long remained underrepresented. The last decade, however, has seen significant progress in our mechanistic understanding of human ABC transporter function through the direct structure determination of many of them (4, 44, 65, 74, 78, 82, 85, 87, 92, 94–96, 105, 115, 117, 118, 120, 122, 127, 134, 135, 139, 141, 145, 146, 149, 160, 162, 163, 165, 170–173, 175, 180, 182, 183). Most significantly,

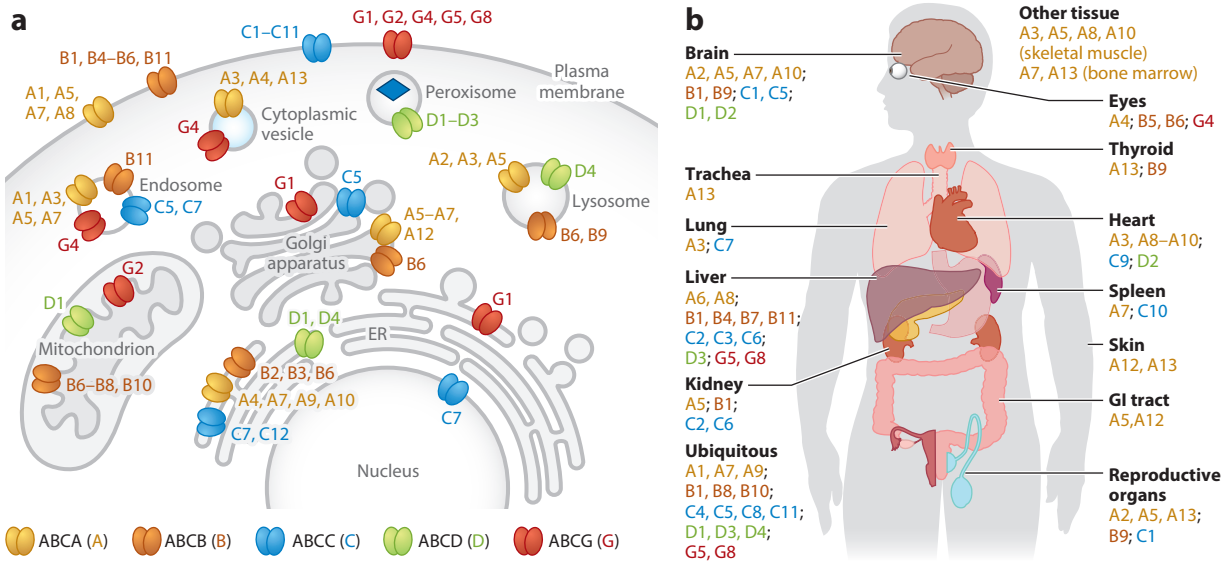



**Figure 1**

Cellular and organ/tissue distribution of human ABC transporters. (*a*) Schematic of human cell showing organellar/plasma membrane distribution of all human ABC transporters. Each subfamily of membrane transporter is depicted in a different color. (*b*) Schematic of human organ system, highlighting the distribution of ABC transporters. Abbreviations: ER, endoplasmic reticulum; GI, gastrointestinal.

**Table 1 Human ABC transporter function and disease association**

| Name (alternate) | Function/substrate/expressing cells/tissue | Mol wt (kDa)/aa | Cellular/organ localization | Disease association |
|---|---|---|---|---|
| ABCA1 (ABC1) | Phospholipid and sterol translocase/lipoprotein biogenesis | 254.3/2,261 | PM, E/ubiquitous | TGD |
| ABCA2 (ABC2) | Probable lipid transporter | 269.8/2,435 | L, E/brain, RO | IDPOGSA |
| ABCA3 (ABC3) | Phospholipid transporter | 191.4/1,704 | L, E, CV/lung, heart, SM | SMDP3 |
| ABCA4 (ABC4) | Retinal–PE flippase, importer | 255.9/2,273 | ER, CV/eyes | STGD1 |
| ABCA5 (ABC5) | Cholesterol efflux transporter; involved in lipoprotein biogenesis | 186.5/1,642 | E, GA, L, PM/RO, GI, brain, SM, kidney | Unknown |
| ABCA6 (ABC6) | Probable lipid transporter | 184.3/1,617 | GA/liver | Unknown |
| ABCA7 (ABC7) | Phospholipid translocator | 234.4/2,146 | PM, GA, E, ER/ubiquitous, brain, spleen, bone marrow | AD9 |
| ABCA8 (ABC8) | Organic cation importer, sterol exporter | 183.7/1,621 | PM/heart, liver, SM | Unknown |
| ABCA9 (ABC9) | Probable lipid transporter | 184.4/1,624 | ER/ubiquitous, heart | Unknown |
| ABCA10 (ABC10) | Probable lipid transporter | 175.8/1,543 | ER/brain, GI, heart, SM | Unknown |
| ABCA12 (ABC12) | Lipid/ceramide transporter | 293.24/2,595 | GA/skin, reproductive organs, GI, brain | ARCI4A |
| ABCA13 (ABC13) | Possible sterol transporter | 576.12/5,058 | CV/bone marrow, trachea, testis, thyroid, lung, skin | Unknown |

(*Continued*)

**Table 1** (*Continued*)

| Name (alternate) | Function/substrate/expressing cells/tissue | Mol wt (kDa)/aa | Cellular/organ localization | Disease association |
|---|---|---|---|---|
| ABCB1 (MDR1) | Translocates drugs and phospholipids across the membrane | 141.47/1,280 | PM | MDR |
| ABCB2 (TAP1) | Peptide transporter. Part of the peptide loading complex. Forms a heterodimer with ABCB3 (TAP2) | 80.96/748 | ER | BLS1 |
| ABCB3 (TAP2) | Peptide transporter. Forms heterodimer with ABCB2 (TAP1) | 75.66/686 | ER/lymph node | BLS1 |
| ABCB4 (MDR3) | Phospholipid transporter | 141.52/1,286 | PM/liver | PFIC3 |
| ABCB5 | Multidrug exporter | 138.64/1,257 | PM/stem-like cells | MDR |
| ABCB6 | Transports a broad spectrum of porphyrins | 93.89/842 | PM, M, E, GA, ER, L/eyes | MCOPCB7 |
| ABCB7 | Exports glutathione-coordinated iron–sulfur clusters | 82.64/752 | M/liver | ASAT |
| ABCB8 | Forms mitochondrial KATP channel | 79.98/735 | M/ubiquitous | ASAT |
| ABCB9 (TAPL) | Broad-spectrum peptide transporter | 84.47/766 | L/testis, brain, spinal cord, thyroid | Unknown |
| ABCB10 | Possible heme transport | 79.14/738 | M/ubiquitous | Unknown |
| ABCB11 (BSEP) | Transports hydrophobic bile salts across the canalicular membrane of hepatocytes | 146.40/1,321 | PM, E/liver | PFIC2 |
| ABCC1 (MRP3) | Broad-spectrum exporter of organic anions, glutathione conjugates, and xenobiotics | 171.59/1,531 | PM/lungs, testis, blood | DFNA77<br>MDR |
| ABCC2 (MRP2) | Transports a wide variety of endogenous compounds, including glucuronide and glutathione conjugates, bile acid conjugates, and xenobiotics | 174.21/1,545 | PM/liver, kidney, intestine | DJS<br>MDR |
| ABCC3 (MRP3) | Transports a wide variety of endogenous compounds, including glucuronide and glutathione conjugates and xenobiotics | 169.34/1,527 | PM/liver | MDR |
| ABCC4 (MRP4) | Transports endogenous metabolites and xenobiotics | 149.53/1,325 | PM (ACM)/ubiquitous, pancreas | MDR |
| ABCC5 (MRP5) | Transports nucleotide and amino acid metabolites | 160.66/1,437 | PM, GA, E/ubiquitous, brain | MDR |
| ABCC6 (MRP6) | Extrudes physiological compounds (glutathione conjugates) and xenobiotics from cells | 164.91/1,503 | PM, ER/liver, kidney | PXE |
| ABCC7 (CFTR) | Transport of chloride ions | 168.14/1,480 | PM, E, ER, N/lungs | CF |

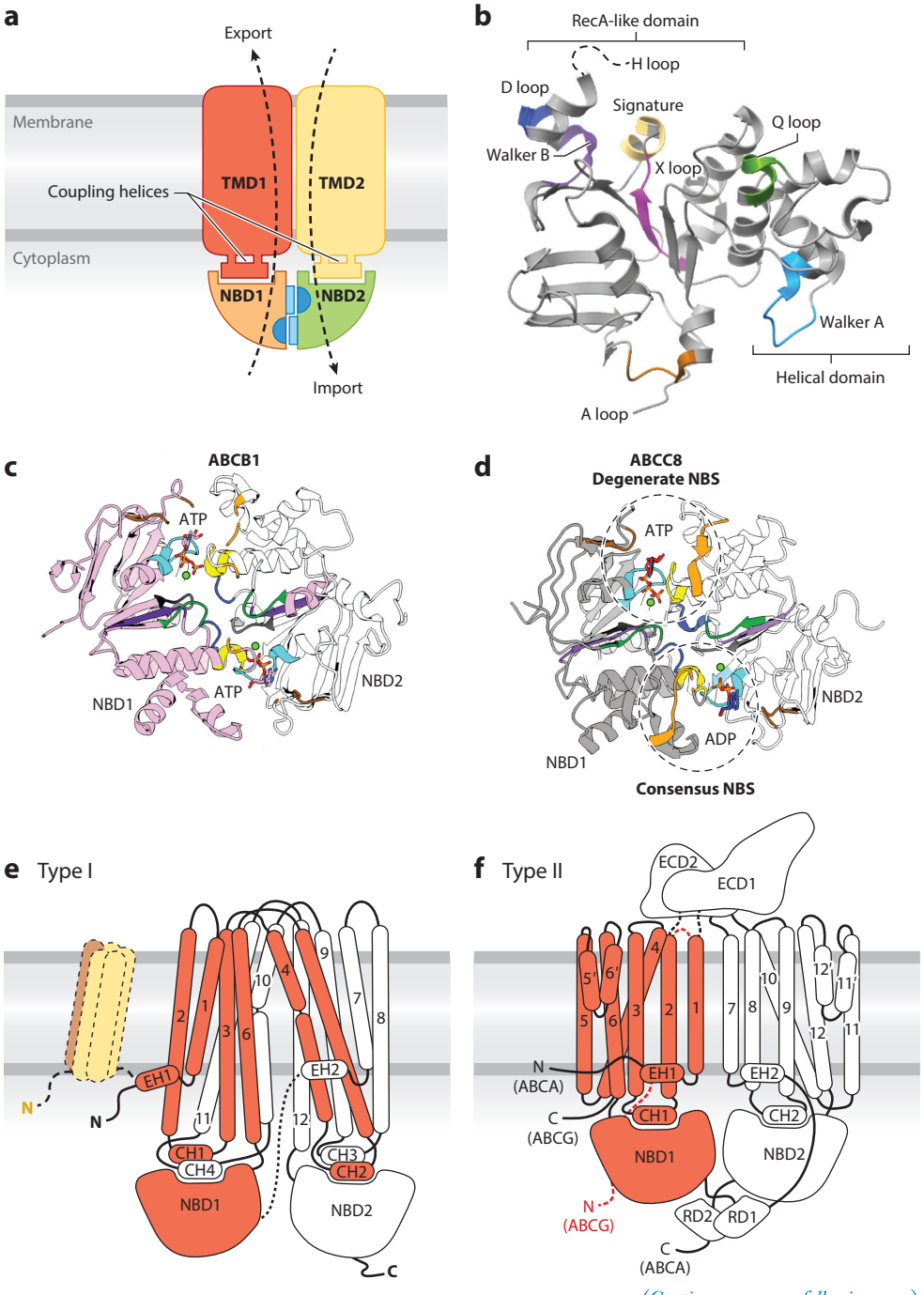
(*Continued*)

**Table 1 (*Continued*)**

| Name (alternate) | Function/substrate/expressing cells/tissue | Mol wt (kDa)/aa | Cellular/organ localization | Disease association |
|---|---|---|---|---|
| ABCC8 (SUR1) | Subunit of the beta-cell KATP channel | 176.99/1,581 | PM/pancreas, enriched, ubiquitous | PNDM3 |
| ABCC9 (SUR2) | Regulatory subunit of the KATP channel with KCNJ11 | 174.22/1,549 | PM/heart | CMD1O |
| ABCC10 (MRP7) | Glucuronide and glutathione conjugate transporter | 161.63/1,492 | PM (ACM)/spleen | MDR |
| ABCC11 (MRP8) | Transports lipophilic anions, steroid sulfates, glucuronides | 154.30/1,382 | PM (ACM)/ubiquitous | MDR |
| ABCC12 (MRP9) | Unknown ligand | 152.30/1,359 | ER/testis | MDR |
| ABCD1 (ALDP) | Transport of VLCFA-CoA from the cytosol to the peroxisome lumen | 82.93/745 | Peroxisome, M, ER, L/ubiquitous | X-ALD |
| ABCD2 (ALDRP) | Transport of VLCFA-CoA from the cytosol to the peroxisome lumen | 83.23/740 | Peroxisome/brain, heart | |
| ABCD3 (PMP70) | LCFA/BCFA, DCA/LCFA-CoA transport | 75.48/659 | Peroxisome/liver, ubiquitous | CBAS5 |
| ABCD4 (PXMP1L) | Cobalamin transporter | 68.59/606 | ER, L/ubiquitous | MAHCJ |
| ABCG1 | Phospholipid transport | 75.59/678 | PM, ER, GA | |
| ABCG2 (BCRP) | Broad-spectrum drug efflux pump | 72.31/655 | PM, M | MDR |
| ABCG4 | Suggested sterol transporter | 71.9/646 | PM, E, CV/brain, eye | |
| ABCG5 | Forms heterodimer with ABCG8. Sterol transport across the cell membrane | 72.50/651 | PM/liver, intestines | STSL2 |
| ABCG8 | Forms heterodimer with ABCG5. Sterol transport across the cell membrane | 75.68/673 | PM/liver, intestines | GBD4, STSL1 |

Abbreviations: ACM, apical cell membrane; AD9, Alzheimer's disease 9; ALDP, adrenoleukodystrophy protein; ALDRP, adrenoleukodystrophy related protein; ARCI4A, ichthyosis, congenital, autosomal recessive 4A; ASAT, anemia, sideroblastic, spinocerebellar ataxia; BCFA, branched chain fatty acid; BCRP, breast cancer resistance protein; BLS1, bare lymphocyte syndrome 1; BSEP, bile salt export pump; CBAS5, congenital bile acid synthesis defect 5; CF, cystic fibrosis; CFTR, cystic fibrosis transmembrane conductance regulator; CMD1O, cardiomyopathy, dilated 1O; CV, cytoplasmic vesicle; DCA, dicarboxylic acid; DFNA77, deafness, autosomal dominant, 77; DJS, Dubin–Johnson syndrome; E, endosome; ER, endoplasmic reticulum; GA, Golgi apparatus; GBD4, gallbladder disease 4; GI, gastro-intestinal tract; IDPOGSA, intellectual developmental disorder with poor growth and with or without seizures or ataxia; KATP channel, ATP-sensitive potassium channel; L, lysosome; LCFA, long chain fatty acid; M, mitochondrion; MAHCJ, methylmalonic aciduria and homocystinuria type cblJ; MCOPCB7, microphthalmia, isolated, with coloboma, 7; MDR, multidrug resistance; MRP, multidrug resistance-associated protein; N, nucleus; PCMP1L, peroxisomal membrane protein 1-like; PE, phosphatidylethanolamine; PFIC2/3, progressive familial intrahepatic cholestasis 2/3; PM, plasma membrane; PMP70, 70 kDa peroxisomal membrane protein; PNDM3, diabetes mellitus, permanent neonatal, 3; PXE, pseudoxanthoma elasticum; RO, reproductive organs; SM, skeletal muscle; SMDP3, pulmonary surfactant metabolism dysfunction 3; STGD1, Stargardt disease 1; STSL1/2, sitosterolemia 1/2; SUR, sulfonylurea receptor; TAP, transporter associated with antigen processing; TAPL, TAP like; TGD, Tangier disease; VLCFA, very long chain fatty acid; X-ALD, X-linked adrenoleukodystrophy.

advances in single-particle cryo-EM analysis have made hitherto intractable targets accessible to high-resolution structure determination. Here we provide an overview of these recent advances, focusing primarily on human ABC transporters for which experimentally determined structures are available at resolutions better than 5 Å (side chain resolution). We summarize the mechanistic insights gleaned from these structures and discuss the limitations of our current state of




(*Caption appears on following page*)

**Figure 2** (*Figure appears on preceding page*)

ABC transporter general architecture. (*a*) Schematic of ABC transporter domain core domain architecture, with arrows indicating the direction of transport. (*b*) NBD from ABCB2/TAP1, with conserved ATPase domains separately labeled and colored. The H loop, missing in this structure, is depicted as a dashed black line. (*c*) Structure of NBD heterodimer from ABCB1 (6C0V) showing two consensus NBSs and bound ATP (*sticks*). (*d*) Structure of NBD heterodimer from ABCC8 (7S5V) showing one consensus NBS with bound ADP and one degenerate NBS with bound ATP. $Mg^{2+}$ ions in panels *c* and *d* are depicted as green spheres. (*e*,*f*) Topology of type I and type II ABC exporters. Half-transporters are shown in red, while full transporters comprise both halves (*red* and *white*). Membrane bilayer is depicted in gray. Dashed lines demarcate TMD0 and altered N-terminal topology of certain type I exporters, with the dotted line denoting the linker region between two halves of a full transporter. For type II exporters, half-transporters of the G subfamily have an NBD first topology and correspondingly altered N and C termini (*dotted red lines*). Similarly, the region between TMD1 and TMD2 diverges between the A and G subfamilies (*dotted black* and *red lines*, respectively). Abbreviations: NBD, nucleotide binding domain; NBS, nucleotide binding site; TMD, transmembrane domain.

knowledge and potential future directions to fill in these gaps in our understanding of human ABC transporters both in terms of fundamental understanding of their physiological functioning and as validated pharmacological and clinical targets.

## NUCLEOTIDE BINDING DOMAINS: STRUCTURALLY CONSERVED MOTIFS FOR ATP HYDROLYSIS

The NBDs remain largely conserved in both sequence and structure in all ABC transporters. The first determined structure of a human NBD was that of ABCB2/Tap1 (transporter associated with antigen processing 1) in complex with ADP (**Figure 2*b***). It displayed a conserved fold that comprises an alpha helical domain, a RecA-like domain, and a beta domain (52) seen in structures of prokaryotic homologs. ATP binding occurs at the interface of two NBDs arranged in a head-to-tail orientation (**Figure 2*c*,*d***), creating two nucleotide binding sites (NBSs) utilizing critical motifs from both NBDs. These include the walker A motif or P loop [consensus sequence G*x*x*x*GK(S/T), with *x* = any residue], which binds the alpha and beta phosphates of ATP; the A loop, which features an aromatic residue that stacks against the purine ring of ATP; the walker B motif (consensus sequence *hhhh*DE, with *h* denoting a hydrophobic residue), which provides the catalytic glutamate residue and aspartate residue to coordinate the required $Mg^{2+}$ ion and contact a bound water molecule that will subsequently attack the γ-phosphate group; the signature motif (consensus sequence LSGGQ), which helps orient the bound ATP molecule; the Q loop, which contacts the TMDs and contains a conserved glutamine that coordinates the bound $Mg^{2+}$ ion; the D loop (consensus sequence SALD), which plays a role in NBD dimerization; the H loop, which features a conserved histidine involved in ATP hydrolysis; and the X loop, which provides contacts between the TMDs in a closed conformation. A subset of ABC transporters has alterations in one of the NBSs, usually in NBD2, which render them competent for ATP binding but not hydrolysis, as recently reviewed elsewhere (144). The physiological relevance of these degenerate NBSs is not fully understood, but they have been proposed to be permanently occupied by a nonhydrolyzed ATP molecule, thereby keeping the NBDs permanently associated and potentially facilitating more efficient ATP hydrolysis at the consensus NBS (153).

## TRANSMEMBRANE DOMAINS: DRIVERS OF STRUCTURAL AND MECHANISTIC DIVERSITY

In contrast to the NBDs, the TMDs of ABC transporters display significant structural differences and are the key drivers of diversity in substrate specificity and transport. Bacteria, archaea, and

**Transmembrane domain (TMD):** the core domain of each transporter half containing multiple transmembrane helices responsible for substrate recognition and transport coupled to nucleotide binding domains

**Helical domain:** a nucleotide binding subdomain found in many ATPases that contains the signature motif

**RecA-like domain:** a nucleotide binding subdomain sharing fold with *Escherichia coli* recA; comprising a central beta sheet flanked by alpha helices

**Nucleotide binding site (NBS):** a site for ATP binding and hydrolysis combining structural elements from both nucleotide binding domains; there are two NBSs per transporter

**Degenerate NBS:** an NBS, generally the first, in some full-length ABC transporters, with alterations rendering it competent for ATP binding but not hydrolysis

fungi express ABC importers and ABC exporters, featuring distinct and characteristic TMD folds (151). In contrast, humans and other higher eukaryotes primarily express ABC exporters that feature two core TMD folds. One of these (referred to as a type I exporter or a type IV transporter) adopts a domain swapped arrangement, and the other (referred to as a type II exporter or a type V transporter) does not, as is expanded upon below. The blueprint for type I ABC exporter architecture was established by the structure of the bacterial multidrug exporter Sav1866 (29) and remains, at its core, conserved among all organisms (**Figure 2*e***). Human type I ABC exporters include members of the B–D subfamilies. The B subfamily contains both half-transporters and full transporters, whereas the C subfamily contains only full transporters and the D family contains only half-transporters. Each type I TMD contains six transmembrane helices (TMs) and two cytoplasmic coupling helices (CHs). CH1 connects TM2 and TM3 (and TM8 and TM9 in full transporters) and contacts NBD1, whereas CH2 connects TM4 and TM5 (and TMs 10 and 11 in full transporters) and contacts NBD2. This domain swapped arrangement couples the conformations of NBDs with the TMDs. The blueprint for human type II ABC exporters, which includes subfamily A and G members, was revealed by the structure of the heterodimeric ABCG5/8 transporter and showed a core TMD comprising six TMs, albeit with a single CH, no TMD domain swapping, and two reentrant helices between TM5 and TM6 (85) (**Figure 2*f***). In comparison to type I exporters, whose TMs extend further below the membrane bilayer, the NBDs of type II exporters sit closer to the lipid bilayer.

**Importer:** a transporter mediating substrate transport toward the cytosol/nucleotide binding domains

**Exporter:** a transporter mediating substrate transport away from the cytosol/nucleotide binding domains; human ABC transporters belong to type I or type II exporter folds

**Domain swapping:** transporter architecture with each transmembrane domain comprising transmembrane helices from both halves of the transporter common to the type I exporter fold

**Extracellular domain (ECD):** an extracellular accessory domain common to ABCA subfamily transporters

**TMD0:** an N-terminal accessory transmembrane domain found in certain ABCB and ABCC subfamily transporters

**Regulatory domain (RD):** a cytosolic accessory domain of either ABCA or certain ABCC subfamily transporters

## ACCESSORY DOMAINS FURTHER DIVERSIFY HUMAN ABC TRANSPORTER STRUCTURE AND FUNCTION

The basic TMD–NBD structural framework described above can be modulated by additional domains that may regulate the function of the NBDs and TMDs, as reviewed previously (14). The accessory domains of human ABC transporters can be classified into extracellular domains (ECDs), N-terminal TMDs (TMD0s), and cytoplasmic regulatory domains (RDs). ECDs exist in all ABCA family members and comprise two parts, ECD1 and ECD2, located in external loops linking TM1 and TM2 or TM7 and TM8, respectively. Each ECD establishes contacts with both TMDs, thereby adding an element of domain swapping, as first revealed by the structure of human ABCA1 (127) (**Figure 2*f***). The ABCA1 ECD comprises three functionally distinct components: the base, tunnel, and lid regions. In ABCA1, the ECD has been suggested to play a role in substrate sequestration and apolipoprotein interactions (45, 164). The ECDs of other ABCA family members vary in size, and their physiological roles remain to be established. ABCA family members also contain a cytosolic RD composed of two halves. Each half shares a similar ACT-like domain fold with a βαββαβ topology (22) and is located immediately after each NBD. The two halves coassemble as a domain swapped dimer, with each half establishing contacts with the NBD of its opposite half (94). Cystic fibrosis transmembrane conductance regulator (CFTR)/ABCC7 also contains an RD of ∼200 residues between NBD1 and TMD2 that has alpha helical character and contains multiple phosphorylation sites. Phosphorylation of the R domain promotes ATP hydrolysis and concomitant channel opening (18). Finally, TMD0s are found in both ABCC (except ABCC7/CFTR) and ABCB subfamily members and are thought to be involved in protein processing, trafficking, and interactions. The TMD0s of ABCC family members contain five TMs, whereas those of Bsubfamily members have four or five TMs and are found only in a subset of half-transporters. In ABCB2/ABCB3 (Tap1/Tap2) and in ABCC8/ABCC9, the TMD0 domains have a role in the formation of oligomeric complexes with associated membrane proteins [e.g., ATP-sensitive potassium (KATP) channels in the case of ABCC8/ABCC9]. The functional roles of other TMD0 domains are less clearly established.

## HUMAN ABC TRANSPORTER FUNCTIONS

Human ABC proteins belong to seven distinct families. Of these, five (subfamilies A–D, and G) comprise membrane transporters that are the focus of this review. The additional ABCE and ABCF subfamilies contain members that lack transmembrane domains and are instead involved in cellular functions such as modulating ribosome activity, as recently reviewed elsewhere (73, 112, 113). They are not discussed further here. Among the human ABC transporters involved in membrane transport, the ABCA subfamily contains 13 genes encoding the largest of all ABC transporters (on average ~2,500 amino acids in length, with the exception of ABCA13, which is purported to be more than 5,000 amino acids long but which remains undetected at the protein level). ABCA members function predominantly as translocators of sterols, lipids, and lipid-like compounds and have been reported to interact directly with apolipoproteins as part of lipoprotein biogenesis pathways. They have remained comparatively understudied, despite their physiological relevance to lipid and cholesterol homeostasis and disease association. Dysfunction of ABCA1, which is involved in translocation of phospholipids and cholesterol to apolipoproteins for high-density lipoprotein (HDL) biogenesis, leads to Tangier's disease (46, 148, 159, 176); this disease is characterized by low plasma HDL levels and buildup of cholesteryl esters, which has severe implications for cardiovascular disease. ABCA2 dysfunction is associated with intellectual and developmental deficiency (102), and transporter activity has been linked to amyloid beta homeostasis, thereby pointing to a potential role in Alzheimer's disease (AD) pathogenicity (28, 107). Genetic variations in the gene encoding ABCA3, a transporter involved in phospholipid transport from the cytoplasm to the lumen of lamellar bodies, can cause pulmonary surfactant metabolism dysfunction 3, a severe respiratory disorder (106, 137, 177). Mutations in the gene encoding ABCA4, a transporter exclusively localized in photoreceptor cells and unique within the subfamily due to its substrate [retinal–phosphatidylethanolamine (PE) conjugates] import functionality, cause Stargardt disease 1, a form of macular degeneration (7, 128, 132, 168). ABCA7 single-nucleotide polymorphisms and coding variants are linked to AD pathogenesis through dysregulated or diminished transporter function (8, 12, 31, 61, 80, 83, 101, 147). This transporter shares functional overlap with ABCA1 and affects phagocytosis and immune response, both contributing factors in AD. Finally, *ABCA12* mutations are linked to defective skin barrier formation and associated abnormalities in harlequin ichthyosis (62, 70, 150).

The ABCB subfamily comprises 10 transporters with widely divergent substrate specificities and includes arguably the most widely studied human ABC transporter, ABCB1 [also known as permeability (p) glycoprotein/P-gp/PGY, or multidrug resistance (MDR) protein 1] (17, 37, 39, 51, 67). ABCB1 is a polyspecific multidrug exporter with a role in cellular detoxification that can be leveraged by cancer cells to acquire MDR in response to chemotherapeutic intervention. The transporter is expressed at several blood–organ barriers, and genetic variants of *ABCB1* have been linked to development of inflammatory bowel disease (9). Clinical targeting of ABCB1 in cancer therapy, however, has proven problematic due to systemic toxicities and off-target effects, and the transporter remains the focus of intense effort for therapeutic/diagnostic development. Its influence on drug-drug interactions and drug pharmacokinetics and disposition has prompted its inclusion in the Food and Drug Administration's list of transporters to screen all new drugs against (154). ABCB2 and ABCB3 (TAP1 and TAP2, respectively) are half-transporters that assemble as a heterodimer that is part of the larger peptide loading complex (PLC) comprising tapasin (81, 123), calreticulin (133), ERp57 (38), and the major histocompatibility complex class 1 heavy chain and β2-microglobulin that transports antigenic peptides from the cytosol to the ER lumen. ABCB4, also known as MDR3 due to its high sequence similarity with ABCB1, is expressed in liver cells and translocates phosphatidylcholine (PC) from the inner leaflet of the

canalicular membrane into bile canaliculi, where PC mitigates the toxicity of bile salts in primary bile (41, 93, 109, 140). Disease-causing variants of the *ABCB4* gene are associated with progressive familial intrahepatic cholestasis 3 (PFIC3), a severe liver disorder (32). ABCB5 is a plasma membrane protein associated with MDR and may act as a regulator of progenitor cell fusion and development of the corneal epithelium (48, 49, 79). Variants of the gene encoding ABCB6, a mitochondrial broad-spectrum porphyrin transporter involved in heme biosynthetic pathways, cause dyschromatosis universalis hereditaria 3 (27, 179) and ocular coloboma (161) and are associated with hemolytic disease in fetuses or newborns (58). ABCB7, also expressed in mitochondria and involved in heme trafficking, is critical for cytosolic Fe–S cluster maturation (174). The inherited X-linked sideroblastic anemia with ataxia is caused by partial inactivating mutations of the *ABCB7* gene (6, 11, 103). ABCB8, which is localized in the inner mitochondrial membrane, also has a role in mitochondrial iron homeostasis and cytosolic Fe–S protein maturation by facilitating mitochondrial iron export (64) and is important in normal heart function (10). It is thought to form a complex with mitochondrial KATP channels to protect cells from oxidative stress (124). Deletion of *Abcb8* in mice led to cytosolic Fe–S cluster deficiency and spontaneous cardiomyopathy (64, 174). ABCB9, also known as TAP-like (TAPL), translocates peptides from the cytosol to the lumen of lysosomes (36, 169, 185). While the exact physiological substrate of ABCB10 is unknown, *ABCB10* knockout mice are embryonic lethal, *ABCB10* overexpression increases hemoglobin synthesis (54, 136), and its genetic variants are associated with type 2 diabetes (104, 110). Finally, ABCB11 (also known as the bile salt export pump) is a bile salt–specific transporter expressed exclusively in the canalicular membrane of hepatocytes (142, 143). Its dysfunction or inactivation by drugs can increase the concentration of bile salts in liver cells and has been associated with PFIC2, a heterogeneous group of rare, autosomal recessive liver diseases in childhood (181).

The ABCC subfamily contains 12 genes encoding for proteins that feature the type I exporter fold. While most of these transporters are collectively classified as MDR-associated proteins (MRPs), this family is notable for the inclusion of three unique members involved in ion transport either by channel activity (ABCC7/CFTR) or by regulating ion transport in the context of a larger complex with inward rectifier potassium channels (ABBC8/SUR1 and ABCC9/SUR2). All ABCC members, with the exception of CFTR, have TMD0 domains that may be involved in protein targeting. ABCC1/MRP1 is of considerable pharmacological and clinical importance as a major player in MDR of cancer cells. ABCC2, also known as canalicular multispecific organic anion transporter 1 (cMOAT1), is involved in canalicular drug transport mostly of conjugated drugs (26, 90), and its genetic variants are associated with Dubin–Johnson syndrome (158), a liver disorder characterized by hyperbilirubinemia. ABCC3 (cMOAT2) shares functional overlap with ABCC2 and is also involved in drug transport (77, 178). ABCC4, ABCC5, and ABCC6 also transport anticancer drugs, although their roles in MDR are comparatively less understood. Mutations in *ABCC6* cause the heritable connective tissue disorder pseudoxanthoma elasticum (13, 84). ABCC7, also known as CFTR, is a chloride ion channel important in fluid homeostasis (129, 131). CFTR mutations can cause cystic fibrosis, a lethal genetic disorder characterized by a failure of mucociliary clearance and eventual respiratory failure (40). ABCC8 and ABCC9 [also known as sulfonylurea receptor (SUR)1 and SUR2, respectively] are components of beta cell and cardiac smooth muscle KATPs, respectively (1–3, 23). Dysfunctional mutants of *ABCC8* are associated with hypoglycemia and hyperinsulism (114, 138, 152), while those of *ABCC9* are associated with cardiomyopathy and other heart defects (15, 56, 121, 155). Both ABCC10/MRP7 and ABCC11/MRP8 (21, 55) are associated with MDR, while the substrate specificity of ABCC12/MRP9 is currently unknown.

The ABCD subfamily contains four half-transporters featuring the type 1 exporter fold. Three of these—ABCD1, ABCD2, and ABCD3—are exclusively localized in peroxisomes and

play a role in the import of various long chain fatty acids (LCFAs) (47, 72, 108, 126, 156, 157). ABCD1 imports very long chain fatty acids and is also known as adrenoleukodystrophy protein since its dysfunction leads to the severe neurological condition X-linked adrenoleukodystrophy (X-ALD) (42, 72, 111). It shares functional overlap with ABCD2, although only mutations in the gene encoding ABCD1 are associated with X-ALD (71). ABCD3 is the most functionally distinct of the three, and its dysfunction has been associated with bile acid synthesis defects (43). The peroxisomal ABC transporters have also been reported to have intrinsic thioesterase activity, which has been linked to their LCFA transport properties, but are generally accepted to bind their substrates in their coenzyme A (CoA) esterified form (30, 108, 156). ABCD4, originally thought to be peroxisomal, is now suggested to be localized in lysosomes and involved in cobalamin/vitamin B12 trafficking out of lysosomes. Mutations in *ABCD4* have been reported to cause vitamin B12 deficiency (24, 35, 68, 69). The topology of all four ABCD family members places both the N and C termini in the cytoplasm, which means that ABCD4 imports vitamin B12 into the cytoplasm, in contrast to its peroxisomal counterparts, which have a canonical exporter functionality.

The ABCG subfamily comprises five genes encoding half-transporters that assemble as homodimers or heterodimers, adopting a type II exporter fold. G subfamily members are the only human ABC transporters that contain an N-terminal NBD followed by a C-terminal TMD (**Figure 2*f***). They are involved either in sterol/lipid transport and homeostasis or in mediation of multidrug transport. ABCG1 transports cholesterol and sphingomyelin and has a role in reverse cholesterol transport from peripheral tissues (75, 176). ABCG2 is a multidrug exporter of extreme physiological importance. Together with ABCB1, ABCG2 forms a pair of drug resistance transporters that act as gatekeepers in the blood–brain barrier. ABCG2 also transports a wide variety of drugs and affects their pharmacokinetics. It has a role in sterol sulfate and urate transport and has been reported to contribute to MDR in various cancer cells (76, 89, 90). Like ABCG1, ABCG4 is thought be involved in sterol transport, and although the two transporters have been proposed to also function as heterodimers, most studies support the notion that both are homodimers (25, 57). In contrast, ABCG5 and ABCG8 form obligate heterodimers that are involved in cholesterol transport from hepatocytes into bile canaliculi (88, 166). Mutations in both genes are related to sitosterolemia, a recessive disorder characterized by drastically elevated plasma sterol levels, by increased sterol absorption, and by poor bile secretion of sterols (63, 100).

## STRUCTURAL BIOLOGY OF HUMAN ABC TRANSPORTERS: CHALLENGES AND RECENT PROGRESS

Human ABC transporters remain challenging targets for high-resolution structural analysis. The heterologous expression of functionally intact human protein samples requires costly eukaryotic expression systems and is further hampered by inherently low yields. In addition, most human ABC transporters display extreme conformational flexibility and heterogeneity and low protein stability. A fundamental complexity in studying human ABC transporters remains the analysis of specific and nonspecific lipid interactions that play important physiological roles but are difficult to analyze in detergent environments commonly used for structural studies. While several membrane reconstitution methods amenable to single-particle cryo-EM analysis have been devised (50, 53, 130), the stable purification of the transporters and the optimization of conditions for stable and pure membrane integration yielding homogeneous samples for downstream structure function studies remain difficult. Furthermore, functional validation of all in vitro model systems is hampered by the difficulties in studying highly hydrophobic substrates such as sterols and lipids. An additional problem is that the resolution required to characterize the interaction of

ABC transporters with bound substrates or ions is often challenging to achieve. Finally, in consideration of the aforementioned conformational heterogeneity, linking observed conformational states to functionally relevant stages of the transport cycle remains difficult. Prior to 2016, only two human ABC transporter structures, which were determined by X-ray crystallography, were reported (85, 135). Since then, advances in instrumentation and data processing software have made human ABC transporters more amenable to intermediate- and high-resolution analysis by cryo-EM. Consequently, rapid progress in structure determination of members of all subfamilies of human ABC transporters (**Figures 3** and **4**) has led to a better understanding of the specifics of their conformational landscape, substrate transport cycles, inhibition, and regulation.

**Inward-facing (IF):** a transporter conformation characterized by separated nucleotide binding domain and transmembrane domain opening to the cytosol

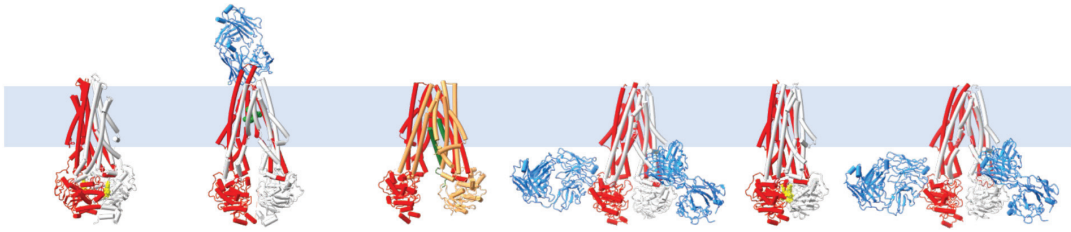
**Outward-facing (OF):** a transporter conformation characterized by dimerized, nucleotide-bound nucleotide binding domains with transmembrane domain opening opposite the cytosol

**Alternating access:** a generalized mechanistic model entailing substrate binding pocket alternating between inward-open and outward-open states during substrate transport

## Conformational Landscape of Human Type I ABC Exporters

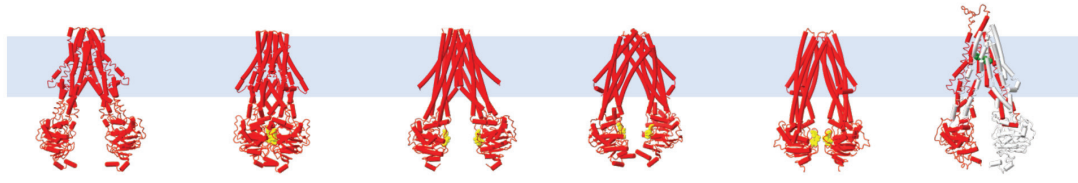
The inward-facing (IF) conformations of most type I exporters in the absence of bound ligands are defined by NBDs separated by various distances and a large V-shaped opening to the intracellular space and inner membrane leaflet. These structures have generally been determined in the absence of bound nucleotides (**Figure 3**), with a few exceptions (173), such as the ABCB7 (cryo-EM), ABCB8 (cryo-EM), and ABCB10 (crystal) structures, that show IF conformations in the presence of bound nucleotides. Ligand- and/or substrate-bound structures of type I exporters have been determined in both IF states and occluded states (**Figure 5*a***). Substrate binding is generally linked to a conformational change bringing the NBDs closer together. IF substrate-bound states have been captured for ABCD1 and ABCB11, whereas occluded states have been captured for ABCB1 and ABCB4. In the case of human ABCB1, substrate- and inhibitor-bound structures have been determined in an occluded conformation with a central ligand binding cavity completely closed off to the bilayer, cytosol, and extracellular space. These states have been captured only in the presence of antigen binding fragments (Fabs) of the externally binding inhibitory antibodies UIC2 and MRK16. Cavity occlusion occurs through a (pseudo)symmetric secondary structure break and kinking of TM4 and TM10 and through gate formation at the cytoplasmic entrance (4, 116). Inhibitors and substrates bind in the same general location within this occluded cavity but display subtle differences in how binding affects the orientation of NBDs; such differences may relate to the divergent roles of inhibitors and substrates in the transport cycle. ABCA4 bound to PC was also captured in an occluded conformation, with a single phospholipid (PC) molecule trapped in the central cavity (115). ABCB11 was captured with both one and two taurocholate molecules, with both structures showing a near-identical transporter conformation (163). The two molecules of C22:0-CoA bound to ABCD1 (20) make contacts with both halves of the transporter, with the CoA moiety associating with one while the acyl chain extends toward and makes contacts with the other. Substrate binding is accompanied by a closing of the inter-NBD gap. In the case of the C18:1-CoA-bound state (165), the NBDs remain further apart, and both the CoA and acyl chains remain associated with the same ABCD1 monomer.

NBD-closed conformations, generally captured using ATP hydrolysis–deficient mutants or nonhydrolyzable ATP analogs, are broadly characterized by a characteristic NBD sandwich dimer with trapped nucleotides in both NBSs. This conformation is accompanied by large-scale structural changes in the TMDs, resulting in either (*a*) an outward-facing (OF) conformation with a V-shaped opening like that seen in ABCD1 and ABCD4 or (*b*) a closed TMD pathway as seen in ABCB1 and ABCB4. The latter have been proposed to be postsubstrate release states. Structural transitions from IF to OF states generally follow established patterns of conserved movements of helix pairs 1/2 (7/8), 3/6 (9/12), and 4/5 (10/11) (86). Overall, the substrate- and ATP-influenced conformational landscape of type I ABC exporters fits with an alternating access model with a central substrate binding cavity exposed to opposite sides of the membrane in IF or OF conformations.



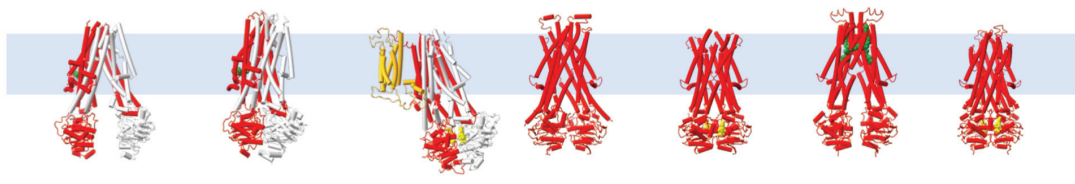
**ABCB1 (6COV)** | **ABCB1 (6QEX)** | **ABCB2/B3 (5UID)** | **ABCB4 (7NIU)** | **ABCB4 (6S7P)** | **ABCB4 (7NIV)**

| | ABCB1 | | ABCB2/B3 | ABCB4 | | |
|---|---|---|---|---|---|---|
| **Conformation** | Closed | Occluded | IF | IF | Closed | Occluded |
| **PDB** | 6C0V | 6QEX*, 7A65*, 7A69*, 7A6F*, 7A6E*, 7A6C*, 7O9W* | 5U1D | 7NIU*, 7NIW* | 6S7P | 7NIV* |
| **Ligand** | | Y (except 7A65) | Y | Y (except 7NIU) | | |
| **Nucleotides** | Y | | | | Y | |



**ABCB6 (7EKM)** | **ABCB6 (7EKL)** | **ABCB7 (7EKL)** | **ABCB8 (7EHL)** | **ABCB10 (4AYW)** | **ABCB11 (7E1A)**

| | ABCB6 | | ABCB7 | ABCB8 | ABCB10 | ABCB11 |
|---|---|---|---|---|---|---|
| **Conformation** | IF | Occluded | IF | IF | IF | IF |
| **PDB** | 7D7R, 7EKM, 7D7N | 7EKL | 7VGF | 7EHL | 4AYT, 3ZDQ, 4AYW, 4AYX | 7E1A, 7DV5, 6LR0 |
| **Ligand** | | | | | | Y (except 6LR0) |
| **Nucleotides** | | Y | | | Y | |



**ABCC7 (7SVR)** | **ABCC7 (7SVD)** | **ABCC8 (7S5V)** | **ABCD1 (7RRA)** | **ABCD1 (7RR9)** | **ABCD1 (7VZB)** | **ABCD4 (6JBJ)**

| | ABCC7 | | ABCC8 | ABCD1 | | | ABCD4 |
|---|---|---|---|---|---|---|---|
| **Conformation** | IF Dephosphorylated | Closed Phosphorylated | Closed | IF | OF | IF | Closed |
| **PDB** | 5UAK, 7SVR | 6MSM, 6O1V, 6O2P, 7SV7, 7SVD | 6C3O, 6C3P, 7S5V, 7S5X | 7RRA, 7SHN, 7VWC | 7RR9, 7SHM, 7VX8 | 7VZB | 6JBJ |
| **Ligand** | Y (except 5UAK) | Y (except 6MSM) | | Y (except 7RRA) | | Y | |
| **Nucleotides** | | Y | Y | | Y | | Y |

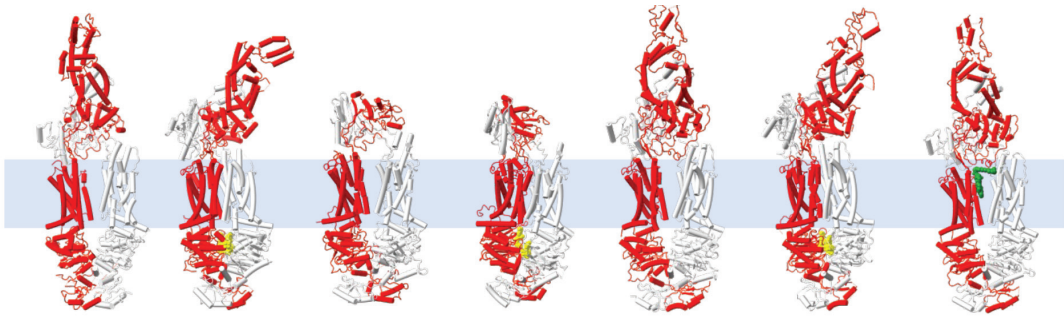
*(Caption appears on following page)*

**Figure 3** (*Figure appears on preceding page*)

Cartoon representation of select type I human ABC transporters with experimentally determined structures. The presence of ligands or nucleotides is indicated with a Y. Fabs are indicated in blue, and Fab complexed structures are denoted by asterisks. The membrane bilayer is depicted as a solid blue bar. Abbreviations: Fabs, antigen binding fragments; IF, inward facing; OF, outward facing; PDB, Protein Data Bank.

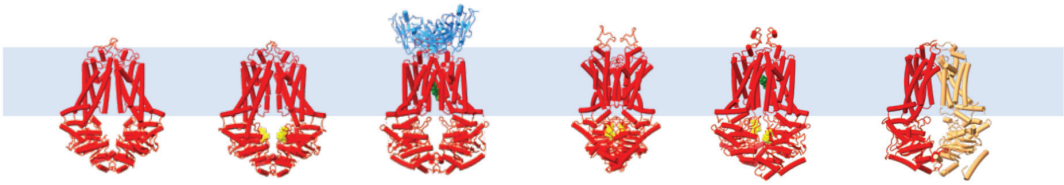
## Conformational Landscape of Human Type II ABC Exporters

Structures of human type II ABC exporters, as for their type I counterparts, have been determined in distinct states including IF and OF states, in line with an alternating access mechanism.



**ABCA1 (5XJY)** | **ABCA1 (7TBW)** | **ABCA3 (7W01)** | **ABCA3 (7W02)** | **ABCA4 (7E7I)** | **ABCA4 (7E7Q)** | **ABCA4 (7E7O)**

| | ABCA1 | | ABCA3 | | ABCA4 | | |
|---|---|---|---|---|---|---|---|
| **Conformation** | Open | Closed | Open | Closed | Open | Closed | Open |
| **PDB** | 7ROQ, 7TC0, 5XJY, 7TBY | 7TBW | 7W01 | 7W02 | 7E7I, 7LKP, 7M1P | 7LKZ, 7E7Q | 7E7O, 7M1Q |
| **Ligand** | | | | | | | Y |
| **Nucleotides** | | Y | | Y | | Y | |



**ABCG1 (7R8D)** | **ABCG1 (7OZ1)** | **ABCG2 (7NEZ)** | **ABCG2 (6HBU)** | **ABCG2 (7OJ8)** | **ABCG5/G8 (5DO7)**

| | ABCG1 | | ABCG2 | | ABCG5/G8 |
|---|---|---|---|---|---|
| **Conformation** | IF | OF | IF | OF | IO |
| **PDB** | 7R8C, 7R8D, 7OZ1 | 7R8E | 6VXI, 6VXJ, 6VXH, 6VXF (closed) 6FFC, 6ETI*, 6HIJ*, 6FEQ*, 7NEQ*, 7NEZ*, 7NFD*, 7OJH | 6HBU | 5DO7, 7R87*, 7R88*, 7R89*, 7R8B* |
| **Ligand** | Y (7R8C) | | Y (except 6VXF) | | Y (7R89, 7R8B) |
| **Nucleotides** | | Y | Y (only 7OJH, 7OJI, 7OJ8) | Y | |

**Figure 4**

Cartoon representation of select type II human ABC transporters with experimentally determined structures. The presence of ligands or nucleotides is indicated with a Y. Fabs are indicated in blue, and Fab complexed structures are denoted by asterisks. The membrane bilayer is depicted as a solid blue bar. Abbreviations: Fabs, antigen binding fragments; IF, inward facing; OF, outward facing; PDB, Protein Data Bank.

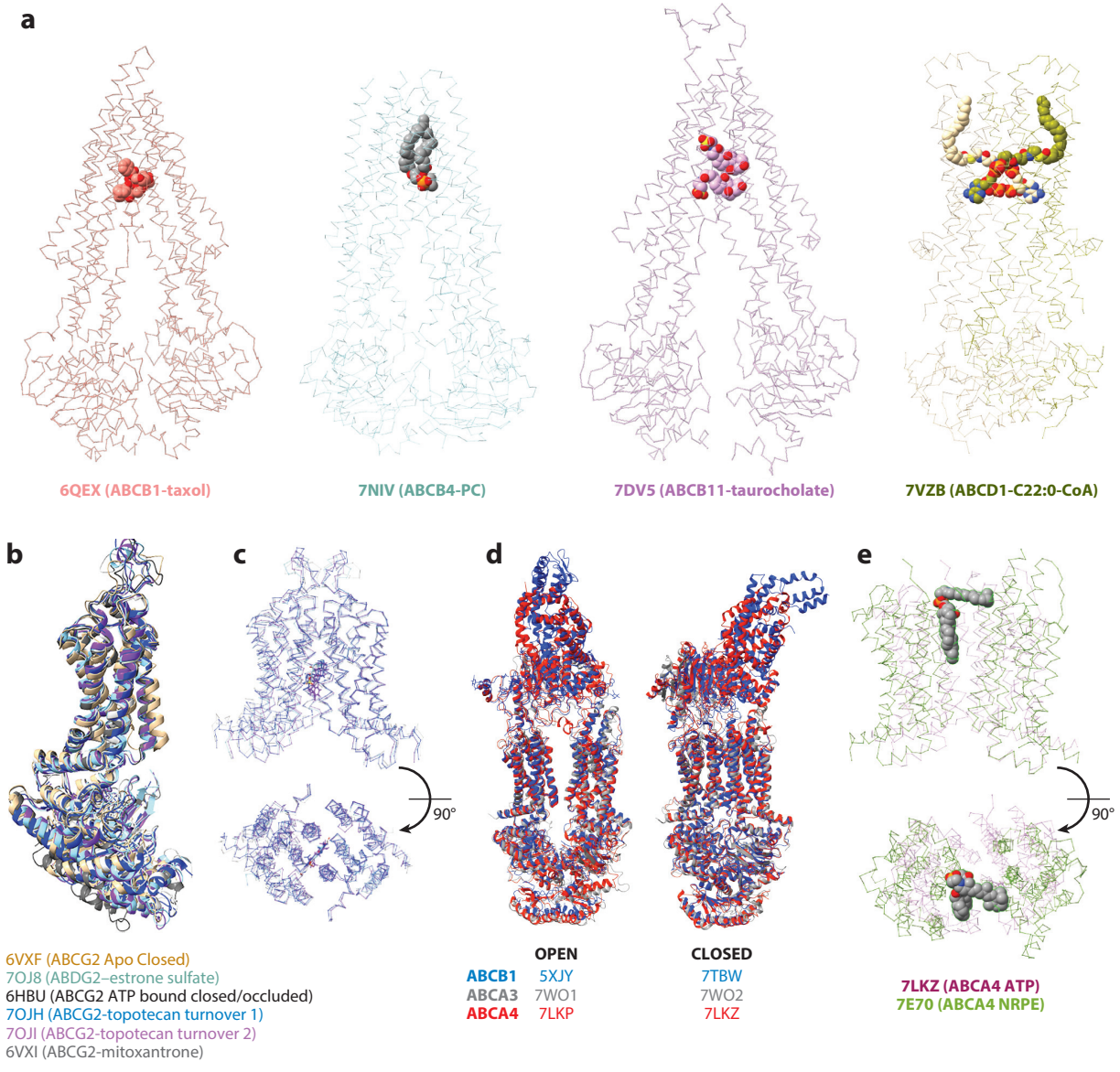



**Figure 5**

Human ABC transporter substrate-bound structures. (*a*) Structures of select type I exporters in complex with substrates (*spheres*). Each ABCD1 half (*far left*) and attached substrates are colored differently. (*b*) Overlaid structures of ABCG2 monomers from different ligand- and/or nucleotide-bound structures aligned using 6HBU chain A as reference. (*c*) Overlay of aligned transmembrane domains of different ligand-bound ABCG2 structures. (*d*) Structures of ABCA1, ABCA3, and ABCA4 in open (*left*) and ATP-bound closed (*right*) states. (*e*) Structures of NRPE-bound ABCA4 (*green*) overlaid with ATP-bound closed ABCA4 (*magenta*). PDB IDs for all structures are shown. Abbreviations: CoA, coenzyme A; NRPE, N-retinylidene-phosphatidylethanolamine; PC, phosphatidylcholine; PDB, Protein Data Bank.

Substrate- and/or inhibitor-bound structures have been captured for ABCA4, ABCG1, ABCG2, and ABCG5/G8. Type II exporters are distinguished from their type I counterparts by smaller substrate binding cavities and greater rigidity of their TMDs, which generally move like rigid bodies as the transporter cycles through different conformations (**Figure 5*b***). However, type II exporters

show a similar pattern of substrate-induced reduction of the inter-NBD distances, with substrates promoting NBD closure in conjunction with nucleotide binding. The most extensive characterization of substrate binding for type II exporters has been of the multidrug exporter ABCG2. IF structures of ABCG2 (**Figure 5*c***) with or without bound substrates have been captured in the presence of a Fab derived from the externally binding, inhibitory IgG-type antibody 5D3 (65, 78, 175, 184). In the absence of 5D3, ABCG2 adopts both an IF (175) and an IF closed state; the latter occurs through a rearrangement of TM2 and TM5, leading to a closure of the intracellular gate, while the binding of inhibitors stabilizes the IF state (122). Under turnover conditions, in which nucleotides and substrates were simultaneously added to wild-type ABCG2, distinct IF conformations, characterized by varying degrees of NBD association, were observed. Finally, the nucleotide-bound state of ABCG2 shows a small external opening referred to as a closed state, with a fully open OF state not yet captured experimentally.

**Lateral access and extrusion:** a substrate translocation model for ABCA family members that is distinct from the alternating access mechanism

A variation of the standard rocker switch-type alternating access model exposing TMD cavities to opposite sides of the membrane in IF and OF conformations has been observed in multiple structures of ABCA family members (**Figure 5*d***). These transporters do not display an IF state akin to those observed in G family transporters. Their open conformation is characterized by a V-shaped opening toward their ECDs, while the nucleotide-bound closed states are characterized by a closed TMD pathway, albeit with a small opening to the extracellular space. A lateral access and extrusion model has been invoked to fit the observed conformations. Structures of ABCA1, ABCA3, and ABCA4 have been captured in multiple states in detergent or lipid environments, while structures with exogenously added substrate have been determined only for human ABCA4, the retinal–PE importer. The substrate binding site in ABCA4 overlaps with the external opening (exit pocket) seen in ABCA family exporters (134, 170). No conformational changes were observed with or without added substrate, and the same mechanism of substrate extrusion from the TMD upon its closure has been evoked for exporters such as ABCA1 and ABCA3 and for the importer ABCA4 (**Figure 5*e***). The details of retinal–PE extrusion into the lower leaflet therefore remain unresolved.

## Structural Insights into Human ABC Transporters Involved in Ion Transport

To date, structures of two members of the ABCC subfamily involved in either direct transport of ions or the modulation of channel proteins have been determined. Multiple structures were reported of human ABCC7/CFTR, the only member of the ABC superfamily that directly transports ions. ABCC7/CFTR is a nonselective anion channel with a TMD ion conduction pathway lined with positively charged residues to aid in recruitment of anions. This purported ion conduction pathway remains open to the cytosol in both the nucleotide-bound and nucleotide-free structures (95, 182), while the conformation captured in the ATP-bound state displays a collapsed TMD pathway unlikely to represent the fully open ion exit pathway (**Figure 6*a***). The current model, based on available structural and functional studies, envisions an unphosphorylated RD wedged between the two CFTR NBDs. Phosphorylation of the RD is thought to lead to its repositioning, which in turn allows for ATP binding and NBD dimerization, triggering channel opening.

The cryo-EM structures of the human pancreatic KATP channel comprising ABCC8/SUR1 and Kir6.2 were recently determined (87, 183), revealing an overall architecture similar to that observed in the previously determined pancreatic KATP channel comprising mouse Kir6.2 and hamster SUR1 (91). As shown in **Figure 6*b***, four subunits, each containing one ABCC8 molecule associated primarily through its TMD0 domain with one Kir6.2 monomer, arrange in a symmetric manner around a central ion conduction pore. Conformational changes of ABCC8 are thought to affect channel opening and closing, although the exact nature of the coupling is not

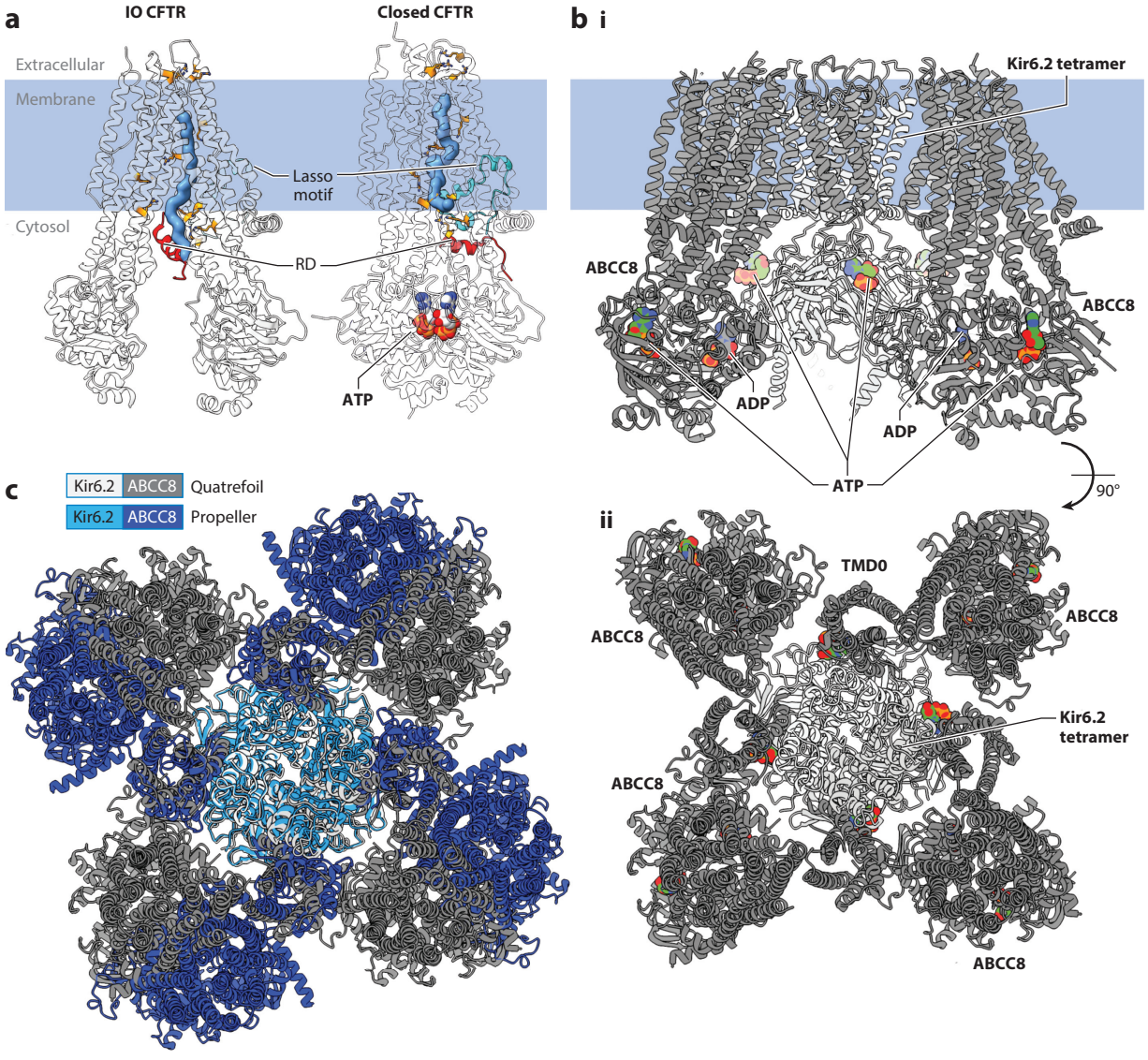



**Figure 6**

Human ABC transporters involved in ion conduction. (*a*) Human CFTR open (*left*) and closed (*right*) structures showing the purported ion conduction pore (*blue* channel) in both states as calculated by MOLEonline (125). Basic residues involved in ion conduction are shown in orange, and part of the R domain and the lasso motif are shown in red and teal, respectively. (*b*) Human KATP channel (63CO) comprising ABCC8 (*dark gray*) and Kir6.2 (*white*) viewed from the membrane plane (*i*) and from the extracellular site (*ii*). Bound nucleotides are indicated as spheres. (*c*) Comparison of quatrefoil and propeller conformations of the human KATP channel (63CO and 63CP, respectively). Abbreviations: CFTR, cystic fibrosis transmembrane conductance regulator; IO, inward open; KATP channel, ATP-sensitive potassium channel; RD, regulatory domain.

fully understood. Currently available human KATP structures have been captured in two main conformations. The quatrefoil conformation is characterized by a narrower diameter and tighter association of the ABCC8 and Kir6.2 subunits, while the propeller form features ABCC8 subunits further apart. In both conformers, ABCC8 was in the NBD-dimerized conformation (**Figure 6*c***).

Interestingly, while ATP-$Mg^{2+}$ was reported to be bound at the degenerate NBS, ADP-$Mg^{2+}$ was found at the consensus NBS, and such localization could be attributed to a higher preference for the diphosphate.

**AlphaFold:** an AI-based program capable of predicting protein structure from its amino acid sequence with high accuracy

## OPEN QUESTIONS AND FUTURE DIRECTIONS IN HUMAN ABC TRANSPORTER RESEARCH

While our understanding of substrate transport cycles and their associated conformational states in human ABC transporters has come a long way, several mechanistic aspects underlying their diverse physiological roles remain unresolved. For example, the role of ECDs in different A subfamily transporters is unclear. There is no clear rationale for potential substrate sequestration by ECDs, as was hypothesized to be relevant for ABCA1 function. For ABCA4, no binding partners have been identified. It is also unclear what mechanisms underlie differential substrate transport directionality by sequentially and structurally related transporters like ABCA1 and ABCA4 or ABCD1 and ABCD4, even though these transporters appear to employ similar ATP-dependent conformational changes. Another area of investigation is our limited understanding of higher-order complexes involving human ABC transporters. Currently, this is limited to initial descriptions of complex architectures (e.g., ABCC8 in KATP and ABCB2/ABCB3 in the PLC), while being completely absent for others (e.g., ABCB8- and ABCB9-based complexes and ABCA family complexes with lipoproteins). Finally, analysis of specific lipid interactions of ABC transporters, in terms of both lipid transport and their regulatory/structural effects, remains challenging. The last few years have also seen rapid advances in structure prediction methods. Specifically, the arrival of AlphaFold (67a) has made the entire human proteome available for three-dimensional analysis. Having the structures of all human ABC transporters available for analysis has high significance for structure-based in silico drug design efforts. However, predicted models of single states currently offer limited information on small-molecule, lipid, and protein interactions.

The recent progress in human ABC transporter structures has revealed the limitations of generalized models in providing detailed mechanistic insights. This highlights the need to work directly on the specific human proteins to realize the true potential of structural biology in bridging fundamental and translational research that may see human ABC transporters increasingly targeted for therapeutic intervention in an ever-growing list of human pathologies associated with their dysfunction.

## DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

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## Errata

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