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MINIREVIEW

Structural snapshot of the cholesterol-transport ATP-binding cassette proteins[1]

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Abstract: The ATP-binding cassette (ABC) proteins play critical roles in maintaining lipid and sterol homeostasis in higher eukaryotes. In humans, several subfamily-A and -G members function as cholesterol transporters across the cellular membranes. Deficiencies of these ABC proteins can cause dyslipidemia that is associated with health conditions such as atherosclerosis, diabetes, fatty liver disease, and neurodegeneration. The physiological roles of ABC cholesterol transporters have been implicated in mediating cholesterol efflux for reverse cholesterol transport and in maintaining membrane integrity for cell survival. The precise role of these ABC transporters in cells remains elusive, and little is known about the sterol-transport mechanism. The membrane constituents of ABC transporters have been postulated to play a key role in determining the transport substrates and the translocation mechanisms via the transmembrane domains. Recent breakthroughs in determining high-resolution structures of the human sterol transporter ABCG5/G8 and its functional homologs have shed light on new structural features of ABC transporters, providing a more relevant framework for mechanistic analysis of cholesterol-transport ABC proteins. This minireview outlines what is known about ABCG cholesterol transporters, addresses key structural features in the putative sterol translocation pathway on the transmembrane domains, and concludes by proposing a mechanistic model of ABC cholesterol transporters.

Key words: ATP-binding cassette, ABCG transporter, cholesterol efflux, transmembrane domain, cardiometabolic disease.

Résumé : Les protéines ABC (« ATP-binding cassette ») jouent des rôles clés dans le maintien de l'homéostasie des lipides et des stérols chez les eucaryotes supérieurs. Chez l'humain, plusieurs membres des sous-familles A et G agissent comme transporteurs de cholestérol à travers les membranes cellulaires. Un déficit en une de ces protéines peut provoquer une dyslipidémie associée à des problèmes de santé comme l'athérosclérose, le diabète, la stéatose hépatique et la neurodégénérescence. Les rôles physiologiques des transporteurs de cholestérol ABC comprennent la médiation de la sortie du cholestérol dans le transport inverse du cholestérol et du maintien de l'intégrité membranaire nécessaire à la survie cellulaire. Le rôle précis de ces transporteurs ABC demeure flou et on connaît peu de choses du mécanisme de transport des stérols. On a postulé que les constituants membranaires des transporteurs ABC jouent un rôle clé dans la détermination des substrats à transporter et des mécanismes de translocation par les domaines transmembranaires. Des percées récentes dans la détermination des structures à haute résolution du transporteur de stérols ABCG5/G8 et de ses homologues fonctionnels ont révélé de nouvelles caractéristiques structurales des transporteurs ABC, fournissant un cadre d'analyse mécanistique plus pertinent des protéines de transport du cholestérol. Cette mini-synthèse met en évidence ce que l'on connaît des transporteurs de cholestérol ABCG, présente des caractéristiques structurales clés des domaines transmembranaires en lien avec le mécanisme possible de translation des stérols et conclut en proposant un modèle mécanistique des transporteurs ABC de cholestérol. [Traduit par la Rédaction]

Mots-clés : cassette de liaison d'ATP, transporteur ABCG, sortie de cholestérol, domaine transmembranaire, maladie cardiométabolique.

Introduction

The ATP-binding cassette (ABC) proteins are ubiquitously present in all phyla of life, representing one of the two largest protein superfamilies conserved across evolution (Higgins 1992), the other one being the major facilitator superfamily (Yan 2015). Most ABC proteins are involved in the primary active transport of solutes such as sugars, amino acids, peptides, ions, and a wide range of hydrophobic compounds across cell membranes. ABC proteins play major physiological roles in almost all vertebrate organ systems (Table 1), and genetic mutations causing malfunction of human ABC proteins have been implicated in diseases (Gottesman and Ambudkar 2001; Borst and Elferink 2002; Moitra and Dean 2011). The ABC proteins consist of transporters and nontransporters. The minimal functional unit of the transporters is composed of two transmembrane domains (TMD1 and 2) and two nucleotide-binding domains (NBD1 and 2). During the transport cycle, the NBDs dimerize to create an interface that can bind and hydrolyze ATP, thereby generating the power stroke to drive active transport of substrates across biological membranes. On the other hand, the nontransporters only include the canonical ATP-binding NBD and usually play important roles in ribonuclease inhibition and translational control (Kerr 2004).

Human ABC proteins are traditionally classified into seven subfamilies (ABCA to G) based on the domain arrangement and full-length sequence homology, including the transporter subfamilies ABCA, B, C, D, and G and the nontransporter subfamilies ABCE and F (Dean et al. 2001; Dean and Annilo 2005; Moitra and Dean 2011).

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Table 1. Human ATP-binding cassette proteins.

	Subfamily	Human ABC proteins	Physiological role (known and probable)	Disease	Structure	Select citation(s)
ABC1	B: multidrug resistance, MDR, 11 members	ABCB1	Efflux of xenobiotics	Multidrug resistance	Kim and Chen 2018	Riordan et al. 1985
		ABCB2	Peptide transport associated with antigen processing	Immune deficiency	Oldham et al. 2016	Deverson et al. 1990; Monaco et al. 1990; Spies et al. 1990; Trowsdale et al. 1990
		ABCB3				
		ABCB4	Phospholipid excretion into bile	Progressive familial intrahepatic cholestasis type III		Van der Bliek et al. 1987
		ABCB5	Efflux of xenobiotics			Allikmets et al. 1996
		ABCB6	Porphyrin transport			Mitsuhashi et al. 2000
		ABCB7	Transport substrate involved in the mitochondrial iron homeostasis	X-linked sideroblastic anemia with ataxia		Savary et al. 1997
		ABCB8	Mitochondrial iron and glutathione export; efflux of xenobiotics			Allikmets et al. 1996
		ABCB9	Peptide translocation to lysosomes			
		ABCB10	Involved in heme biosynthesis		Shintre et al. 2013	Zhang et al. 2000
		ABCB11	Bile salt secretion into bile	Progressive familial intrahepatic cholestasis type II		Strautnieks et al. 1998
	C: multidrug resistance-associated protein, MRP, 12 members	ABCC1	Multispecific organic anion transport	Multidrug resistance	Martin et al. 2017*a*	Cole et al. 1992
		ABCC2	Renal and biliary elimination of organic anionic substrates	Dublin–Johnson syndrome		Büchler et al. 1996
		ABCC3	Organic anion transport			Kiuchi et al. 1998
		ABCC4	Nucleotide transport; antiviral drug efflux			Kool et al. 1997
		ABCC5	Nucleotide and glutamate conjugate transport			Jedlitschky et al. 2000; Wijnholds et al. 2000
		ABCC6	Transport of organic anions	Pseudoxanthoma elasticum		Kuss et al. 1998
		ABCC7	Epithelial chloride channel	Cystic fibrosis; congenital bilateral absence of the vas deferens	Liu et al. 2017	Riordan et al. 1989
		ABCC8	Modulation of associated potassium channels	Hyperinsulinemic hypoglycemia of infancy	Martin et al. 2017*a*	Aguilar-Bryan et al. 1995
		ABCC9		Cantu syndrome		Chutkow et al. 1996
		ABCC10	Efflux of xenobiotics			Allikmets et al. 1996
		ABCC11	Anionic hydrophobic solute transport	Resistance to anticancer and antiviral nucleoside based drugs		Lagasse and Clerc 1988
		ABCC12	Unknown			Tammur et al. 2001
	D: adrenoleukodystrophy-related protein, ALD, 4 members	ABCD1	Long and very long chain fatty acid transport	Adrenoleukodystrophy		Mosser et al. 1993
		ABCD2				Holzinger et al. 1999
		ABCD3	Branched chain fatty acid transport	Zellweger syndrome		Kamijo et al. 1990
		ABCD4	Possible role in vitamin B12 transport			Holzinger et al. 1997
ABC2	A: 12 members	ABCA1	Cholesterol and phospholipid transport	Tangier disease; familial high-density lipoprotein deficiency	Qian et al. 2017	Luciani et al. 1994
		ABCA2	Phospholipid transport			
		ABCA3	Phospholipid transport	Neonatal surfactant deficiency		Connors et al. 1997
		ABCA4	Transport of retinoid	Stargardt macular degeneration; cone–rod dystrophy		Allikmets et al. 1997
		ABCA5	Nucleotide and glutamate conjugate transport			Arnould et al. 2002
		ABCA6	Role in macrophage lipid homeostasis			Kaminski et al. 2001
		ABCA7	Phospholipid and sphingolipid transport			Kaminski et al. 2000

Table 1 (*concluded*).

Subfamily	Human ABC proteins	Physiological role (known and probable)	Disease	Structure	Select citation(s)
G: five members	ABCA8	Cholesterol and taurocholate transport	Harlequin ichthyosis	Taylor et al. 2017	Arnould et al. 2002
	ABCA9	Role in macrophage lipid homeostasis			Piehler et al. 2002
	ABCA10	Role in macrophage lipid homeostasis			Wenzel et al. 2003
	ABCA12	Sphingolipid transport			Annilo et al. 2002
	ABCA13	Unknown			Prades et al. 2002
	ABCG1	Cholesterol and phospholipid transport			Chen et al. 1996; Savary et al. 1996
	ABCG2	Efflux of xenobiotics	Multidrug resistance		Allikmets et al. 1998; Doyle et al. 1998; Miyake et al. 1999
	ABCG4	Cholesterol transport			Annilo et al. 2001; Oldfield et al. 2002
	ABCG5 ABCG8	Cholesterol and plant sterol efflux	β-Sitosterolemia	Lee et al. 2016	Berge et al. 2000
E: one member	ABCE1	Role in translation initiation and ribosome recycling		Preis et al. 2014; Shao et al. 2016†,‡	Wolkoff et al. 1985
F: three members	ABCF1	Regulation of innate immune response			Richard et al. 1998
	ABCF2	Role in cell volume regulation			Allikmets et al. 1996
	ABCF3	Probable role in cell proliferation			

Note: The 48 human ABC proteins from the subfamilies ABCA–G can be classified into two groups, ABC1 and ABC2, adapted from the classification based on TMD origin by Wang et al. (2009) and Zheng et al. (2013). Physiological function and disease phenotypes were obtained from www.genecards.org and https://ghr.nlm.nih.gov/. Structural information is presented for human orthologs, except*, †, and ‡, which indicate bovine, yeast, and rabbit homologs, respectively.

Recent analysis on the membrane constituents of the ABC exporters indicated that at least three separate events led to the evolution of the TMDs and thus reclassified the ABC efflux transporters into three groups: ABC1, ABC2, and ABC3 (Wang et al. 2009; Zheng et al. 2013). In humans, ABCB/C/D and ABCA/G belong to ABC1 and ABC2 porters, respectively (Table 1). The phylogenic analysis suggests that although the NBDs may have evolved once (monophyletic), at least three separate events led to the evolution of the TMDs (polyphyletic) followed by the TMD–NBD fusion (Xiong et al. 2015).

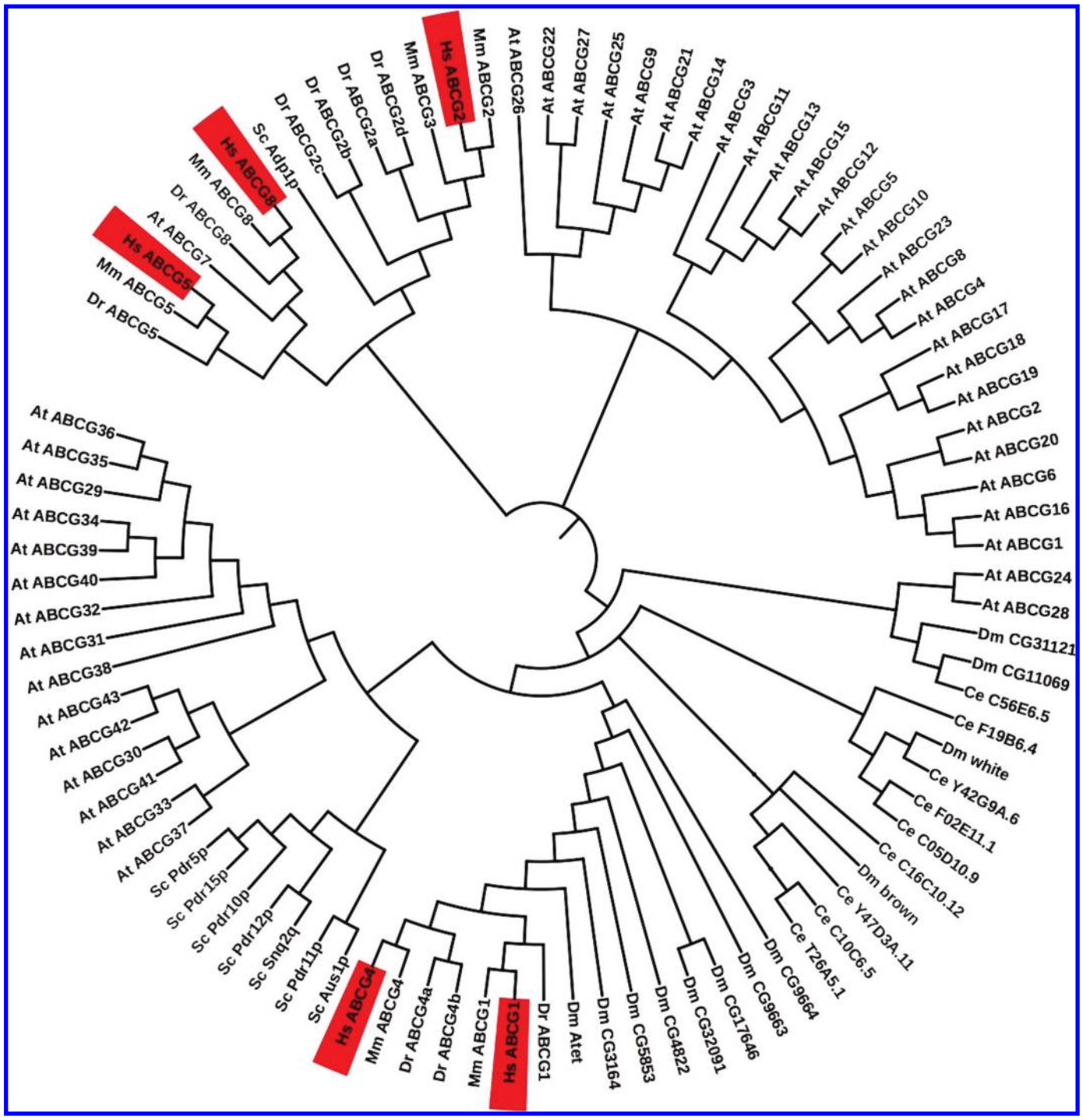
During the last two decades, structural, biochemical, and biophysical studies have shed light on the functions and molecular mechanisms of ABC proteins (for extensive reviews: Jones and George 2004; Hollenstein et al. 2007; Linton 2007; ter Beek et al. 2014; Beis 2015; Wilkens 2015; Locher 2016). Advancement of structural biology has enriched our molecular understanding of the functions and mechanisms of ABC transporters. However, it was not until recently that ABCG transporters and an ABCA protein revealed a TMD fold that is unique to the ABC2 exporters (Lee et al. 2016; Qian et al. 2017; Taylor et al. 2017), whereas previously known ABCB or ABCC transporters share a similar TMD structural fold of the ABC1 superfamily (Aller et al. 2009; Oldham et al. 2016; Zhang and Chen 2016; Johnson and Chen 2017, 2018; Martin et al. 2017*a*, 2017*b*). In this minireview, we will focus on the ABCG transporters that are implicated mainly in cholesterol homeostasis in the body. In light of the recent structural information of the ABC2 members, i.e., ABCG5/G8 (Lee et al. 2016), ABCG2 (Taylor et al. 2017), and ABCA1 (Qian et al. 2017), the main objective is to provide a mechanistic basis of ABC transporter-mediated cholesterol transport by interpreting recent biochemical and structural data of the cholesterol-transport ABC proteins.

ABCG transporters

In comparison with other ABC protein members, the subfamily-G transporters are unique in their domain arrangement: an N-terminal NBD followed by a C-terminal TMD (Dean et al. 2001). In eukaryotes, ABCG proteins are either half or full transporters, i.e., the half transporters form homo- or heterodimeric complexes as the minimal functional unit and the full transporters are primarily found in fungi and plants. So far, five members of ABCG transporters have been discovered in the human genome: ABCG1, ABCG2, ABCG4, ABCG5, and ABCG8. Using the TMD defined by the crystal structure of human ABCG8 as the reference, we analyzed the amino acid sequence homology of the ABCG transporters from seven representative eukaryotes: human (*Homo sapiens*), mouse (*Mus musculus*), zebrafish (*Danio rerio*), thale cress (*Arabidopsis thaliana*), fruit fly (*Drosophila melanogaster*), round worm (*Caenorhabditis elegans*), and yeast (*Saccharomyces cerevisiae*). As summarized in Fig. 1, human ABCG transporters are clustered in three groups: (1) ABCG1 and ABCG4, (2) ABCG2, and (3) ABCG5 and ABCG8. This is consistent with the previous CLANS (cluster analysis of sequence) analysis using ABCG5 as the template (Lee et al. 2016). Interestingly, the full transporter homologs, e.g., yeast Pdr or AtABCG30–43, are closer to human ABCG1 and ABCG4, while the plant half transporters (AtABCG1–28) are similar to ABCG5 and ABCG8 or ABCG2. This suggests that the evolutionarily closer ABCG transporter homologs may share a similar mechanism or recognize/transport substrates with similar physical and (or) chemical properties.

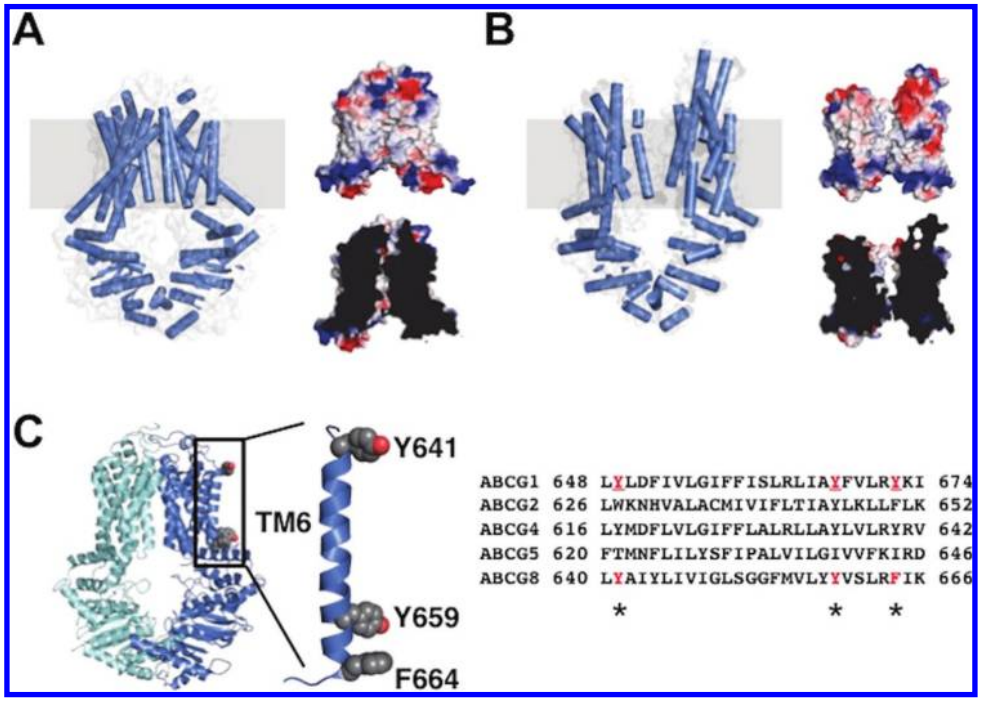
Human ABCG transporters are mostly associated with lipid and sterol metabolism with the exception of ABCG2 that effluxes a wide range of hydrophobic xenobiotics (Table 1). ABCG1 and ABCG4 are believed to translocate cholesterol in the plasma membrane and endosomes (Sano et al. 2014; Pandzic et al. 2017) and play a key role in regulating cholesterol balance in the brain and macrophage-rich tissues, e.g., lung (Savary et al. 1996). ABCG2 is widely expressed in various tissues, where its expression is considered as a marker for stem cells (Maliepaard et al. 2001; Ding

Fig. 1. Transmembrane domain based phylogenetic tree of eukaryotic ABCG transporters. Eukaryotic ABCG homologs were identified using BLAST (basic local alignment search tool) using the transmembrane domain sequence of human ABCG8 as query. The search was limited to model organisms: *Homo sapiens* (Hs) (human), *Mus musculus* (Mm) (mouse), *Danio rerio* (Dr) (zebrafish), *Drosophila melanogaster* (Dm), *Arabidopsis thaliana* (At), *Caenorhabditis elegans* (Ce), and *Saccharomyces cerevisiae* (Sc) (yeast). Redundant sequences were manually excluded. Multiple sequence alignments were performed using COBALT (constraint-based multiple alignment tool). The multiple sequence alignment was used to determine phylogeny using the simple phylogeny (ClustalW2) tool and subsequent tree building was performed with the interactive tree of life web tool (EMBL). Human ABCG transporters are highlighted in red. [Color online.]



et al. 2010) and responsible for the efflux of xenobiotics, cancer drug resistance, and uric acid export (Woodward et al. 2009; Szafraniec et al. 2014). However, cholesterol is required for the transport activity of ABCG2 on the plasma membranes (Telbisz et al. 2007, 2013). ABCG5 and ABCG8 are exclusively expressed in the liver and the small intestines, where they function as heterodimers for cholesterol and plant sterol export into the bile (Berge et al. 2000; Lee et al. 2001; Lu et al. 2001).

Fig. 2. Structural models of human ABC cholesterol transporters. (A and B) Left: Crystal structure of the heterodimeric ABCG5/G8 (A: Protein Data Bank ID 5DO7) and cryo-EM structure of ABCA1 (B: Protein Data Bank ID 5XJY) are depicted in a cylindrical cartoon presentation (marine-blue cylinders) that is embedded by partially transparent surface presentation (white). The cytoplasmic C-termini and the extracellular domain of ABCA1 are omitted. Grey rectangular background: membrane lipid bilayers. Right: Electrostatic surface presentation shows the hydrophobic transmembrane domain boundaries (top, white: hydrophobic, red and blue: charged). TMD cross sections show that both inward- and outward-facing conformations exist in the nucleotide-free apo state of the transporters. (C) Based on the secondary structures of human ABCG5 and ABCG8, sequence alignment was performed using PROMOL3D for human ABCG1, ABCG2, ABCG4, ABCG5, and ABCG8. On the transmembrane helix 6 (TM6), Y641, Y659, and F664 of ABCG8 (red, asterisks) correspond to Y649, Y667, and Y672 of ABCG1 (red, underlined), which were previously shown as a sterol-sensing cholesterol recognition amino acid consensus motif in ABCG1 (Sharpe et al. 2015).



Physiological roles of ABCG sterol transporters

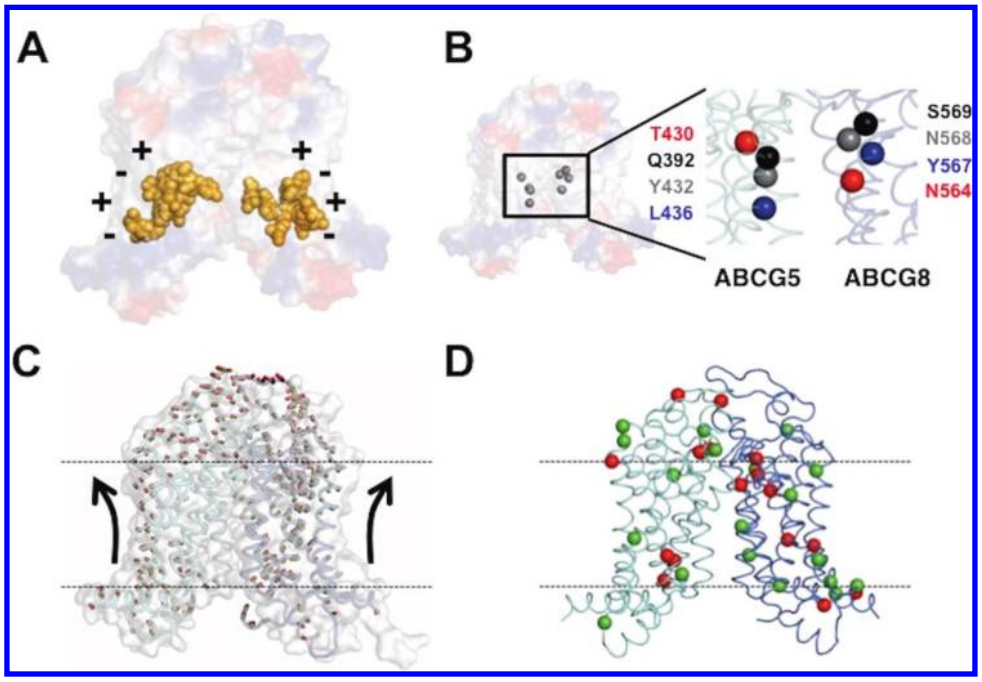
ABCG1 facilitates cholesterol removal from tissues via reverse cholesterol transport and promotes maturation of the circulating high-density lipoprotein particles (Tall et al. 2008). ABCG1 works synergistically with ABCA1 to promote cholesterol efflux (Gelissen et al. 2006; Yvan-Charvet et al. 2007) and to prevent atherosclerosis, a major cause of cardiovascular diseases. Inhibition of ABCG1 reduces efflux of cholesterol and choline phospholipids from the circulating macrophages (Klucken et al. 2000; Kennedy et al. 2005). In mice fed a high-cholesterol and high-fat diet, *Abcg1* deficiency caused lipid accumulation in macrophages and hepatocytes and induced formation of plaque foam cells that led to atherosclerosis (Baldán et al. 2006; Yvan-Charvet et al. 2007). Besides extracellular efflux of cholesterol, ABCG1 has also been shown to export sphingomyelin in an in vitro cell-based assay (Sano et al. 2007), and both lipids are able to stimulate the ATPase activity of ABCG1 (Hirayama et al. 2013).

In addition to lipid efflux, ABCG1 also plays an important role in maintaining cellular structures. ABCG1 has been shown to localize at the junction between plasma membranes and intracellular endosomes and is able to mobilize reorganization of cholesterol and sphigomeylin from ordered to disordered membranes to promote cholesterol efflux (Tarling and Edwards 2011; Neufeld et al. 2014). In pancreatic β-cells, ABCG1 is present on the intracellular insulin granules where it plays a role in the maintenance of the granule morphology and the activity of insulin secretion from the pancreas (Sturek et al. 2010). These results illustrate the emerging roles of ABCG1 in cardiometabolic functions by regulating cholesterol and phospholipid homeostasis and in cell viability by maintaining lipid composition in the cells.

Both ABCG1 and ABCG4 are expressed in the brain, where ABCG1 facilitates cholesterol efflux from astrocytes to extracellular apolipoproteins and lipoproteins and ABCG4 is responsible for cholesterol efflux from neurons (Chen et al. 2013). Expression of mouse Abcg4 in the blood–brain barrier restricts the brain entry of amyloid-β peptide (Aβ), and in vivo studies using Abcg1-null mice have shown increased Aβ secretion (Sano et al. 2016; Dodacki et al. 2017). The underlying mechanism has been recently implicated by the suppression of γ-secretase activity and the reduction of amyloid precursor protein processing (Sano et al. 2016). Because production of Aβ is involved in the pathogenesis of Alzheimer's disease, ABCG1 and ABCG4 can thus serve as targets to treat this neurodegenerative disease.

Heterodimeric ABCG5/G8 complexes are expressed in hepatocytes and enterocytes, where plant sterols and cholesterol are excreted into the bile and gut lumen, respectively. ABCG5/G8 regulates cholesterol metabolism by promoting excretion of hepatic cholesterol into the bile and reducing cholesterol absorption in the gut (Hazard and Patel 2007). Patients with mutations in either gene develop sitosterolemia, a rare autosomal recessive disease characterized by increased plasma levels of plant sterols, hypercholesterolemia, and premature coronary heart disease (Berge et al. 2000; Lee et al. 2001; Lu et al. 2001). Studies on missense mutations have shown the chaperone-driven regulation for the maturation of ABCG5/G8 complexes in the endoplasmic reticulum (Graf et al. 2004). ABCG5/G8 deficiency leads to sterol accumulation and increased fatty acid uptake in cells and tissues (Yu et al. 2002). Both subunits display catalytic asymmetry in regulating the biliary sterol transport as demonstrated using a knockout mouse model of ABCG5/G8 and recombinant adenovirus carrying

Fig. 3. Transmembrane domain structural features in ABCG cholesterol transporters. (A) Sphere presentation (yellow) of the transmembrane domain polar relay residues (+/−) indicates the polar cluster near the cytoplasmic end of the transmembrane domain hydrophobic boundary. (B) Using ABCG5 and ABCG8 as templates, four pairs of amino acids (color-coded in the blowup) are coevolved residues that are separated >8 Å in the crystal structure but predicted to interact upon allosteric changes of the transmembrane domain conformations. (C) Normal-mode molecular dynamics simulation shows projections of upward movement of the transmembrane helices (green-to-red bars), leading to an outward-facing conformation (arrows). According to methods described in Lee et al. (2016), the lowest 20 vibrational modes were obtained, and modes 9 and 13 were selected for further examination. (D) Using the transmembrane domain of heterodimeric ABCG5/G8, red spheres indicate the localization of pathogenic residues whose disease-causing mutations were observed in sitosterolemia patients. Green spheres show the ABCG5 and ABCG8 residues that are equivalent to the pathogenic amino acids in ABCA1. A list of equivalent residues is summarized in Table 2.



the catalytic mutants of ABCG5 or ABCG8 (Zhang et al. 2006). In high-fat diet fed mice, expression of ABCG5/G8 may prevent hepatic fat accumulation by reducing cholesterol concentration and fatty acid uptake (Su et al. 2012). Absence of ABCG5/G8 is associated with hepatic steatosis, i.e., lipid accumulation in the liver, and its inhibition is involved in the development of nonalcohol fatty liver disease in the pathogenesis of obesity (Miettinen et al. 2006; Van Rooyen et al. 2011). In addition, ABCG5/G8 is believed to participate in transintestinal cholesterol (or sterol) excretion (van der Velde et al. 2008; Patel et al. 2018). Together, ABCG5/G8 is a promising drug target for treatment of hepatic and cholestatic disorders.

Structural biology of ABC cholesterol transporters

The ABC cholesterol transporters are members of the ABCA and ABCG subfamilies. Two recent breakthroughs have shed light on the molecular details of cholesterol transporters: the crystal structure of human ABCG5/G8 (Lee et al. 2016) and the cryo-EM structure of human ABCA1 (Qian et al. 2017). Both transporters showed the same structural fold for the TMD, representing common transmembrane porters of the ABC2 superfamily (Figs. 2A and 2B). Both structures were determined in a nucleotide-free apo state. However, the TMD in ABCG5/G8 revealed an inward-facing conformation (Fig. 2A), whereas ABCA1 showed an outward-facing conformation (Fig. 2B). Amino acid sequence alignment of the human ABCG transporters indicated conserved tyrosine and (or) other aromatic residues on the transmembrane helix 6 of ABCG1, 2, 4, and 8 but not ABCG5 (Fig. 2C). In ABCG1, these residues were previously shown to be the cholesterol recognition amino acid consensus motif and responsible for sterol sensing (Sharpe et al. 2015). This therefore suggests that ABCG cholesterol transporters may use this motif to monitor the membrane cholesterol level, and ABCG8 may function as the sterol-sensing subunit for ABCG5/G8-mediated biliary sterol excretion.

Table 2. Transmembrane domain (TMD) pathogenic residues in cholesterol efflux ABC transporters.

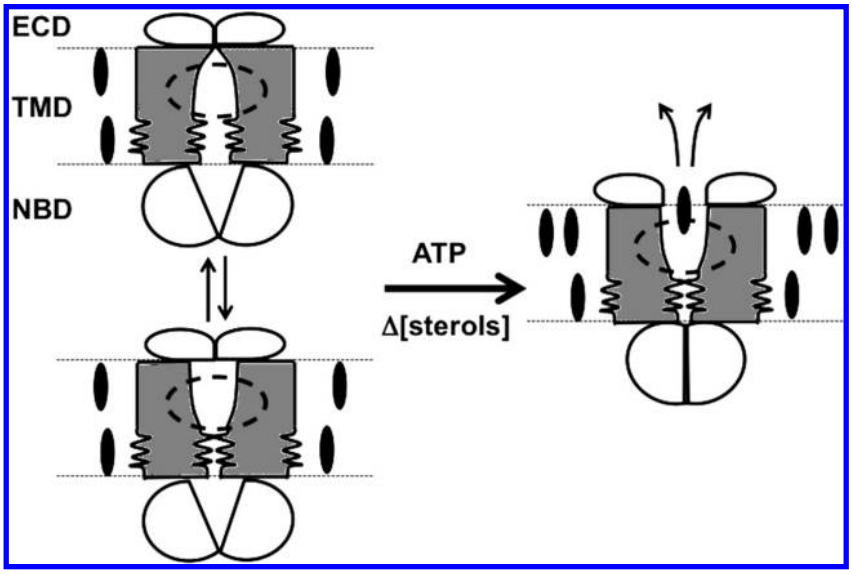
ABCG5/G8[a]		ABCG5/G8 residues equivalent to ABCA1 residues[b]		ABCA1 (limited to ABCG5/G8 equivalents)[b]	
ABCG5	ABCG8	ABCG5/TMD1	ABCG8/TMD2	TMD1	TMD2
R389	T400	I416	R404	M636	R1341
R419	R405	D418	F453	R638	C1660
N437	L501	V420	F461	M640	A1670
I523	R543	V439	L465	I659	V1674
R550	L572	Y458	C471	D677	R1680
C600	G574	S563	G490	V771	W1699
Q604	G575	S618	A495	S824	V1704
M622	L596	R619	I497	V825	D1706
		L634	R522	W840	S1731
			V593		N1800
			I643		R1851

[a]Summarized based on the crystal structure of human ABCG5/G8 (Lee et al. 2016) and a review by Patel et al. (2018).

[b]Summarized based on the cryo-EM structure of human ABCA1 (Qian et al. 2017).

Several TMD structural features are revealed in the apo ABCG5/G8 structure. First, a cluster of polar amino acids, embedded in the middle of the TMD, forms a polar relay near the cytoplasmic end (Fig. 3A). This polar relay is also observed in ABCG2, and mutations of selected residues have been shown to affect

Fig. 4. A working model of the cholesterol efflux mechanism by ABCG transporters. In the nucleotide-free state (open nucleotide-binding domain (NBD)), inward- and outward-facing transmembrane domain (TMD) (grey) conformations exist in equilibrium, while the extracellular domain (ECD) remains closed. The TMD polar relay (zigzags) may reduce the rigidity of the transmembrane helical bundles, allowing a flexible volume between adjacent TMDs to accommodate sterols or other hydrophobic molecules. In response to sterol concentration changes and ATPase activity, the NBD closes, the ECD opens, sterol asymmetry occurs, and the outward-facing TMD engages cholesterol efflux by releasing the sterols to the extracellular acceptors. Black solid oval, cholesterol; dashed open oval, sterol-binding site.



ATPase and drug-transport activities of ABCG2 (Khunweeraphong et al. 2017). Currently, it is unknown how the polar relay impacts the cholesterol-transport function. Speculatively, the polar region may provide the TMD more flexibility to undergo conformational changes during the catalytic and transport cycle. Further studies are needed to address its function.

Second, coevolution analysis of the TMD in ABCG5/G8 identified four pairs of amino acids that are >8 Å away in the nucleotide-free structure (Fig. 3B). Each pair is predicted to engage in close contact during the transport activity, and an in vivo study showed the inhibition of biliary cholesterol secretion by one mutant transporter (Lee et al. 2016). Third, we have previously shown an upward movement of the TMD in ABCG5/G8 by molecular dynamics simulation. In Fig. 3C, two modes of simulation suggest that the current inward-facing conformation of the TMD can project towards an outward-facing conformation under the current nucleotide-free condition. As shown in ABCA1 (Fig. 2B), an outward-facing TMD is possible without ATPase activity. Four, we localize several pathogenic residues on the TMD of ABCG5/G8 whose mutations cause sitosterolemia as well as mapping the equivalent residues to the pathogenic amino acids in ABCA1 (Fig. 3D; Table 2). These residues are mostly localized in or near the area of the polar relay, co-evolution pairs, or the vestibules on the transporter TMD surface (i.e., sterol-binding site). Using an ABCG4 homology model, recent studies showed that ABCG4 interacts with sterols or Aβ at the sterol-binding site (Dodacki et al. 2017).

Mechanistic model of ABCG-mediated cholesterol efflux

Recent studies on ABCG1 and (or) ABCA1 demonstrated that asymmetric distribution of cholesterol in lipid bilayers is generated by the cholesterol-transport activity of proteins leading to cholesterol sequestration to the outer leaflet of cellular membranes (Liu et al. 2017). We speculate that this sterol gradient may be detected by the sterol-sensing motif in the ABC transporters, a similar mechanism proposed for the cholesterol-sensing proteins in the endoplasmic reticulum, e.g., SREBP cleavage-activating protein (Radhakrishnan et al. 2004). Together with the structural analysis, we propose a working model to describe the mechanism of ABC transporter-mediated cholesterol transport (Fig. 4). In the nucleotide-free apo state, the TMDs can undergo inward- and outward-facing conformations in equilibrium, while the ABCs of the NBDs remain open and the extracellular domains stay closed. Upon ATP-induced NBD closure and formation of a cholesterol gradient in the lipid bilayers, the TMD remains in the outward-facing conformation and the extracellular domain opens to release sterol molecules to the sterol acceptors. The polar relay may serve to maintain the flexibility of the TMD as well as allow the transporters to adapt to changing membrane properties resulting from cholesterol asymmetry in the cellular membranes.

Concluding remarks

Human ABCG transporters were discovered in the mid-1990s (Chen et al. 1996; Savary et al. 1996), and in his seminal work over a century ago, Thomas Morgan described the *White* and *Brown* genes encoding the *Drosophila* homologs and established the foundation for modern molecular genetics (Morgan 1910). Over the past two decades, the physiological roles of several ABCG transporters have been defined, but still little is known about the molecular basis of their transport mechanisms. The cholesterol-transport ABC proteins play essential roles in cardiometabolic physiology and in cell survival. It is now clear that the subfamilies G and A share a common structural fold in their transmembrane domains. The atomic- or near atomic-level resolution of ABCG transporters has revealed new details describing their structural impact on transport functions and opens new avenues and challenges for further biochemical and biophysical studies of cholesterol transporters. Future directions seeking to address the structure–function relationship of the cholesterol-transporting ABC proteins will include examining how catalysis is coupled to sterol transport, understanding what determinants control the lipid translocation and recognition, determining the precise role of the extracellular domains, and clarifying how transporter activities respond to lipid metabolism and energy homeostasis at both the cellular and the whole-body levels. With the maturity of membrane protein structural biology and its complementary na-

ture to other biophysical approaches and chemical biology, we are poised to discover more molecular events underlying the mechanism of the physiologically important cholesterol-transport ABC proteins, ultimately enabling structure-based drug discovery and therapeutics for cardiometabolic conditions.

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