Cryo-EM structure of lipid embedded human ABCA7 at 3.6Å resolution

Le Thi My Le^{1†}, James R. Thompson^{1†}, Tomonori Aikawa², Takahisa Kanikeyo² & Amer Alam^{1,*}

¹The Hormel Institute, University of Minnesota, Austin, Minnesota 55912

²Department of Neuroscience, Mayo Clinic, Jacksonville, Florida 32224

[†]These authors contributed equally to this work

*Address correspondence to: Amer Alam, PhD, aalam@umn.edu

Abstract

Dysfunction of the ATP Binding Cassette (ABC) transporter ABCA7 alters cellular lipid homeostasis and is linked to Alzheimer's Disease (AD) pathogenesis through poorly understood mechanisms. Here we determined the cryo-electron microscopy (cryo-EM) structure of human ABCA7 in a lipid environment at 3.6Å resolution that reveals an open conformation, despite bound nucleotides, and bilayer lipids traversing the transmembrane domain (TMD). We show that ATP hydrolysis in ABCA7 is modulated by its lipid environment as well as apolipoprotein (apo) A1 and apoE, the latter in an isoform dependent manner, and that apoA1 can directly bind ABCA7. Structural similarities between ABCA and ABCG family transporters suggest that TMD-nucleotide binding domain (NBD) pairs in both sets move as single rigid bodies to affect conformational transitions. Our data suggest these transitions in ABCA7 are influenced by TMD cavity lipids and apolipoprotein binding in addition to being coupled to ATP hydrolysis.

Introduction

AD accounts for the overwhelming majority of dementias, affecting more than 5.5 million people in the United States alone. In the absence of a cure, this number is expected to increase rapidly, with devastating healthcare and socioeconomic consequences (Alzheimer's Association, 2020). Genome wide association studies have identified *ABCA7* as an important genetic risk factor for AD. *ABCA7* single nucleotide polymorphisms and coding variants are linked to AD pathogenesis through dysregulated or diminished transporter function (Almeida et al., 2018; Bellenguez et al., 2017; De Roeck et al., 2019; Hollingworth et al., 2011; Lambert et al., 2013; Le Guennec et al., 2016; Ma et al., 2018; Swerdlow, 2016). Targeting *ABCA7* may therefore offer a new way forward to facilitate development of novel AD therapeutics. To this end, understanding the molecular details of *ABCA7* lipid interactions, its substrate transport cycle and its regulation, and the physiological consequences of *ABCA7* dysfunction is a fundamental prerequisite for realization of *ABCA7* directed AD therapeutic regimens.

ABCA7 encodes a 2146 amino acid membrane transporter that is expressed in low abundance in many tissues and blood (Iwamoto et al., 2006; Sleegers, 2020). It bears similarities to the phospholipid and sterol transporter *ABCA1*, with which it shares ~54% sequence similarity and functional overlap, as well as the retinal importer *ABCA4*. The latter is functionally distinct from *ABCA7* and *ABCA1* with an opposite direction of substrate transport. All three are expressed as single polypeptides comprising two non-identical halves. Each half consists of a TMD, with the first two transmembrane helices (TMs) of each separated by a large extracellular domain (ECD), and an NBD attached to a cytoplasmic regulatory domain (RD) of largely unknown function (Qian et al., 2017). *ABCA7* and *ABCA1* are distinguished from each other by several functional differences. First, they have reportedly different phospholipid specificities (Quazi and Molday,

2013; Tomioka et al., 2017). Second, while the role of ABCA1 in the formation of high-density lipoprotein (HDL) particles is firmly established, HDL formation by ABCA7 has only been shown in transiently transfected cells and the resulting HDL particles are reportedly smaller than those produced by ABCA1 with a reduced cholesterol content. While ABCA7 therefore serves as a useful model for studying ABCA transporter and apolipoprotein interactions, its physiological role in cellular HDL generation is as of yet unclear. Third, ABCA7 and ABCA1 expression are inversely regulated by cellular cholesterol, with the former downregulated by cholesterol accumulation and the latter upregulated (Ikeda et al., 2003; Iwamoto et al., 2006). Moreover, only ABCA1 dysfunction has been conclusively linked to hypercholesterolemia and atherosclerosis (Meurs et al., 2012; Voloshyna and Reiss, 2011). Finally, both transporters share sequence similarities with the *C. elegans* cell corpse engulfment protein (ced-7: 24% identical to ABCA7), which regulates phagocytosis during programmed cell death (Wu and Horvitz, 1998). However, only depletion or dysfunction of ABCA7 impairs phagocytosis, which is linked to AD through a role in amyloid beta and tau plaque clearance, in mouse fibroblasts, macrophages, and microglia (Abe-Dohmae, 2012; Tanaka et al., 2010).

While the structures of detergent purified ABCA1 and ABCA4 in a nucleotide free and ATP bound conformation have been determined (Liu et al., 2021; Qian et al., 2017), no ABCA7 structure is currently available. The TMD conformation in both nucleotide free structures showed a transmembrane cavity open to the outside with extraneous density purportedly from ordered phospholipids or detergent molecules seen close to the cytoplasmic gate. While these structures have greatly advanced our understanding of the overall architecture of ABCA family transporters, the key question of what the TMDs looks like in a lipid environment remains open. Moreover, there is currently no consensus on how apolipoproteins affect conformational changes, if any, to

influence the lipid transfer activity of ABCA7 and ABCA1. To shed light on these mechanistic aspects of ABCA7 functioning, we purified human ABCA7 from human embryonic kidney (HEK) cells, reconstituted it in phospholipid nanodiscs, and carried out its structural and functional characterization. Our results allow us to begin addressing the influence of lipids and apolipoproteins on ABCA7, providing a general mechanistic framework applicable to other ABCA family transporters, many of whose functions remain understudied at the molecular level. These results open the door for new directions for investigating a functional link between ABCA7 and apoE isoforms and will be of significant interest to medicinal chemists and computational biologists targeting ABCA7 for *in silico* studies.

Results

Phospholipids and apolipoproteins modulate ABCA7 *in vitro* activity

We expressed human ABCA7 harboring a C-terminal yellow fluorescent protein (YFP)-rho-ID4 tag in a tetracycline inducible stable HEK293 cell line. This system has reproducibly yielded high quality preparations of functional ABC transporters devoid of contaminating phosphatases (Alam et al., 2018; Nosol et al., 2020; Olsen et al., 2020). Purified ABCA7 was reconstituted in phospholipid nanodiscs and its ATP hydrolysis activity was analyzed (Figure 1A-C, Figure S1A-B). The nanodisc platform provides a more physiological *in vitro* system for studying integral membrane proteins including other ABC transporters in multiple conformations and allowed us to mimic the expected native membrane bilayer environment of ABCA7 and control its lipid and cholesterol composition. As seen in Figure 1A, nanodisc reconstituted ABCA7 displayed a basal ATPase rate of $\sim 30 \text{ nmol min}^{-1} \text{ mg}^{-1}$ in nanodiscs comprising a mixture of Brain Polar Lipids and Cholesterol (BPL/Ch). ATPase rates were highest in nanodiscs enriched in brain polar

phosphatidylethanolamine (PE), followed by phosphatidylserine (PS) and phosphatidylcholine (PC). Removal of cholesterol led to an increase in ATPase rate, in agreement with previous analyses of liposome reconstituted ABCA7 (Quazi and Molday, 2013). ATP hydrolysis in nanodiscs, liposomes, and detergents followed Michaelis-Menten reaction kinetics with Michaelis constant (K_M) values of 0.4 mM, 0.8mM and 0.8 mM, respectively (Figure 1B). ATPase activity was inhibited by both sodium orthovanadate and the non-hydrolysable ATP analog ATP gamma S (γ S) and stimulated by 2,4-diamino-6,7-dimethoxyquinazoline (DADMQ), a metabolite identified from a metabolomics screen comparing wildtype and ABCA7 heterozygous knockout mice (Figure S1B) (unpublished data). This suggests modulation of ABCA7 activity by not only lipids and sterols, but also select small molecules, and the possibility of DADMQ being an ABCA7 agonist.

Cells expressing ABCA7 have been shown to directly bind apoA1 (Wang et al., 2003), although the exact nature of these interactions is poorly understood. We compared the ATPase activity of detergent purified ABCA7 in the presence of a molar excess of human apoA1, apoE2, apoE3 or apoE4. As shown in Figure 1C, all four recombinant apolipoproteins stimulated ATPase activity above that of detergent solubilized ABCA7 alone. This suggests a direct interaction between the apolipoproteins and ABCA7 that leads to conformational changes resulting in a rearrangement of the NBDs to facilitate ATP hydrolysis. This ATPase rate stimulation is reduced for the E2 variant and almost abolished for the E4 variant, suggesting an altered binding interaction between the apoE isoforms and ABCA7. It is interesting to note that while the apoE4 variant is correlated with increased AD risk and the E2 variant is considered protective, both appear to have a weaker interaction with ABCA7 based on ATPase stimulation. To confirm a direct interaction between ABCA7 and apolipoproteins, we immobilized his-tagged apoA1 on Nickel- nitrilotriacetic acid

(Ni-NTA) resin in the presence of detergent solubilized ABCA7. As seen in Figure 1D, ABCA7 is retained and subsequently co-elutes with apoA1, providing qualitative confirmation of direct complex formation that we speculate holds true for apoE isoforms as well.

Cryo-EM structure of ABCA7 reveals lipid filled TMD cavity

The cryo-EM structure of nanodisc reconstituted ABCA7 was determined to 3.6Å resolution in the presence of ATPγS (Figure 1E, Figure S1C-E). Despite the addition of nucleotide, ABCA7 was seen to adopt an open conformation with separated NBDs similar to that seen for apo human ABCA1 and ABCA4, which we speculate arises from interactions between TMD2 and the ECD as explained below. However, unlike the ABCA1 and ABCA4 structures, the central cavity between TMD1 and TMD2 of ABCA7 reveals density features consistent with a single file of bilayer phospholipids traversing the width of the TMDs (Figure 1F). While we cannot ascertain the lipid identities, their specific orientation, nor exclude the presence of other sterols or lipidic species, likely due to the mobile nature of the observed membrane patch that leads to transient occupancies, these density features are distinguishable from the more delocalized and discontinuous density of the surrounding nanodisc lipids and characterized by 1) a gap between the upper and lower leaflet density features and 2) a gap between the two acyl chains of a single file of phospholipids in both leaflets (Figure 1F, Figure 2A-B). The phospholipids appear continuous with the surrounding membrane except at the intracellular gate formed by residues L655 and T1646 from TM5 and TM11, respectively (Figure 2B). TM1, TM2, and TM5 from TMD1 contribute the majority of residues within 5 Å of the observed phospholipids compared to only TM11 from TMD2 (Figure 2C). This asymmetric arrangement with a smaller TMD2 interface

arises from a rigid body shift of the TMD2-NBD2 pair of ABCA7 compared to the more symmetric arrangement seen in the ABCA1 structure, as expanded upon below.

Details of TMD-ECD and lipid facing interfaces of ABCA7

What leads to the observed asymmetry in TMD arrangement in ABCA7 compared to apo ABCA1 and ABCA4? As seen in Figure 3, the electrostatic potential maps of cavity facing interfaces in ABCA7 reveal marked differences between the two TMDs. The TMD1 interface is significantly more electropositive than that of TMD2 (Figure 3A-B), particularly along the cytoplasmic and extracellular boundaries where cavity facing residues are likely to be in close proximity to any TMD resident lipids. In contrast, the less electropositive TMD2 interface comprises a series of negative charges on its extracellular boundary. This may serve to repel phospholipid headgroups and could account for the increased conformational mobility of TMD2 in the presence of TMD phospholipids. In addition to TMD1, the base region of the ECD comprises a cluster of positively charged residues (Figure 3C), continuous with TMD1. An analysis of the TMD-ECD interfaces (Figure 3D) reveals much more extensive contacts between the ECD and TMD1 (buried surface area of $\sim 790 \text{ \AA}^2$) compared to TMD2 ($\sim 330 \text{ \AA}^2$ buried surface area). Both TMD1 and TMD2 make contacts with the opposite ECD subunits, leading to a domain swapped arrangement that may be important for coupling interdomain conformational changes. The TMD1-ECD2 interface ($\sim 565 \text{ \AA}^2$) is significantly larger than that of TMD1-ECD1 ($\sim 223 \text{ \AA}^2$) and both interfaces comprise an extensive network of polar and electrostatic interactions. Positively charged residues from the ECD located above the TMD cavity are oriented towards the upper leaflet, part of a cluster of positive charges from ECD1, ECD2, and TMD1 that could promote passage of phospholipids to the ECD from the TMD.

Our ABCA7 structure allows us to map known missense ABCA7 variants and also compare known ABCA1 missense variants to analyze conservation in ABCA7. As shown in Figure 4, many pathogenic missense ABCA7 mutants associated with higher AD risk, as well as three ‘protective’ missense variants reported to reduce AD risk are found distributed over the entire ABCA7 structure. Many of these overlap with residues comprising the TMD-ECD interfaces. Within the ECD, three neighboring residues map locations of homologous ABCA1 missense mutants associated with Tangier’s Disease that all reduce ABCA1 interaction with apoA1 as measured using cross-linking ability with both phospholipid- and water-soluble agents (Fitzgerald et al., 2002). Due to the structural homology between ABCA7 and ABCA1, we believe these residues loosely demarcate a putative interface that both transporter proteins use when interacting with apolipoproteins in order to initiate phospholipid efflux from cells to create HDL. In fact, several known pathogenic mutations in ABCA1 that may have a similar effect in ABCA7, based on conservation of the associated residues, are clustered in this region of the ECD above the TMD cavity. On the cytoplasmic side of the transporter, of particular interest is the VFVNFA motif in ABCA1 (Fitzgerald et al., 2004) that has been shown to be essential for phospholipid efflux and apoA1 binding. The equivalently placed VFLYFS motif in ABCA7, while not identical, may play a similar role, suggesting a possible conserved role for the ABCA family RDs in coupling long range conformational changes with the ECD as discussed below. Finally, EM density likely arising from bound phospholipids or cholesterol was also observed in the hydrophobic tunnel within the ECD (Figure 3E), similar to observations from the structure of ABCA1 (Qian et al., 2017). The tunnel has two possible portals that are solvent accessible and was previously suggested to be a temporary space to sequester phospholipids in ABCA1 or serve as a passage for phospholipid transport to apolipoproteins, a postulate supported by our observations in ABCA7.

The observed ECD lipids are predominantly located within ECD1, which contributes the majority of residues within 5Å of them.

Conserved rigid body motions define ABCA7 conformational transitions

Differences in local EM density quality in our ABCA7 maps (Figure S2) suggest increased motion in TMD2 and NBD2, while TMD1-NBD1 and the ECD appear to be more rigid. This is further evidenced by a comparison of our ABCA7 structure with that of ABCA1. As shown in the superposition in Figure 5A, TMD1-NBD1 and the ECD base region display a high degree of structural similarity. However, the TMD2-NBD2 pair undergoes a rigid body shift, involving an outward translation and rotation to a wider open conformation. This leads to an opening of the intracellular gate, marked by the increase in C alpha distances between the two intracellular gate forming residues L655 and T1646 (Figure 4B-C). Interestingly, this shift is also accompanied by a rigid body movement of the entire regulatory domain (RD), which moves as a single unit despite comprising two halves sequentially far apart (Figure 4D). Whereas the RD of ABCA1 maintains molecular interactions with both NBD1 and NBD2, the ABCA7 RD separates from NBD2 while maintaining contact with NBD1. Despite the overall structural change affected by these conformational transitions, individual TMD-NBD pairs and RD share virtually identical architectures, further highlighting that rigid body movements involved (Figure S4).

To establish whether the rigid body motions outlined above extend to other ABC transporters of the Type-V fold (Thomas et al., 2020), we extended our analysis of individual TMD-NBD pairs to members of the G family for which structural data is available, namely ABCG2 and ABCG5/G8 (Figure 6A). As shown in Figure 6B, TMD-NBD pairs from both the apo open (Orlando and Liao, 2020) and ATP bound closed conformation of ABCG2 (Manolaridis et al., 2018) share a virtually

indistinguishable conformation (Figure 6B, left), also shared by ABCG5 and ABCG8 (Lee et al., 2016) (Figure 6B, center). ABCG and ABCA members share mirrored topologies with the former arranged in an NBD-TMD configuration compared to TMD-NBD for the latter. However, the individual TMD-NBD pairs from ABCA7 share strong structural similarities with those of ABCG2 (Figure 6B, right). This suggests the possibility of ABCA family transporters undergoing the same conformational transitions seen in the ABCG family (Figure 6C). The physiological implications of this are expanded upon below.

Discussion

Our data address several unknowns about the physiological functioning of ABCA7. First, we show that the large TMD cavity seen in the open conformation of ABCA7 is completely lipid filled. The uninterrupted passage of phospholipids from the upper leaflet of the surrounding membrane through the ABCA7 TMD raises the possibility that phospholipid efflux, and therefore ABCA7's role in phospholipid homeostasis, may vary by cell type and be influenced by inherent asymmetry in phospholipid distribution between the two bilayer leaflets. By breaking the bilayer's cytoplasmic phospholipid leaflet, TM5 and TM11 may facilitate the extrusion of phospholipids from the upper leaflet of ABCA7. Positively charged cavity facing interfaces of TMD1 and its ECD interface may aid lipid accessibility to the cavity and, subsequently, to the ECD base region for sequestration in the hydrophobic ECD tunnel.

Second, our comparison of ABCA and ABCG family transporters shows that transporters in these families may share common TMD-NBD cores that move in tandem as rigid bodies to affect overall conformational changes. This is unlike type-IV exporters such as members of the ABCB and ABCC families that 1) have a domain swapped architecture and 2) undergo rearrangements of individual TMs and TM pairs during the transport cycle (Lee et al., 2014). This

leads us to propose that the ABCA7 TMD-NBD pairs of ABCA7 may follow a similar set of movements associated with nucleotide dependent NBD dimerization and suggest the possibility of an ABCA7 conformation similar to that seen in the ATP bound ABCG2_{EQ} structure. The transition to a closed ABCA7 structure would therefore only entail rigid body movements of the two halves of the protein barring steric hindrance from the ECD or RD (Figure 6C).

Based on the smaller ECD and RD interfaces involved, we postulate that the TMD2-NBD2 pair is the mobile rigid body unit involved in open to closed transitions in ABCA7. This would entail some form of interfacial disengagement of the ECD from one or both of the TMDs, allowing the TMDs to approach each other in the closed state similar to those of ABCG2. One possible way this could be accomplished would be the binding of apolipoproteins that may cause conformational changes promoting NBD closure. This is further supported by the observation that the hydrolysis deficient EQ mutant of ABCA4 adopts a closed conformation with bound ATP that is accompanied by a reconfiguration of the ECD and TMD2 interface. We show that the ATPase activity of ABCA7 is enhanced in the presence of apolipoproteins, suggesting a direct interaction that may contribute to structural changes in the ECD that are coupled to NBD arrangement. This is in line with data suggesting apoA1 mediated structural changes in ABCA1 (Nagao et al., 2012) and further evidenced by the fact that despite the clear indication of bound nucleotides, we were unable to resolve 3D classes with dimerized NBDs in our EM datasets, which would require rearrangements of the TMD-ECD interface.

Inter-domain rigidity appears to play a key role in relaying long range conformational changes in ABCA7. Individual TMD-NBD pairs move in concert to control access to a central phospholipid cavity. ECD1 and ECD2 both maintain contacts with the opposing TMDs, adding an

element of domain swapping that could play a role in coupling the two halves of the protein. Similarly, while the RD appears to move as one rigid unit, RD1 makes contacts with both NBDs, possibly further contributing to both halves of the transporter being conformationally coupled. The VFLYFS motif in RD2 (equivalent to VFVNFA in ABCA1) shown to affect phospholipid translocation may aid in stabilizing the TMD2-NBD2 pair. In line with this, the RD configuration seen in ABCA1 and ABCA4 structures adopts more symmetric contacts with the two NBDs, with the two halves of ABCA1 and ABCA4 consequently appearing more homogenous in terms of their local structure. While the intracellular gate in our ABCA7 structure adopts a more open conformation compared to ABCA1 and ABCA4, the difference may be a consequence of the absence of phospholipids in the latter two.

Taken together, our data allow us to put forward a hypothetical model for how apolipoprotein-mediated structural changes in ABCA7 and, by extension, ABCA1, may occur (Figure 6D). In the absence of apolipoproteins, ABCA7 adopts an open configuration with a large, central lipid filled cavity open to both bilayer leaflets. The TMD-ECD interfaces in this state afford enough flexibility to allow for a TMD configuration similar to that seen in the open conformation of detergent purified ABCA1 and ABCA4 (dashed lines in figure 6D). The binding of apolipoproteins to ABCA7 may rearrange the ECD and its TMD interactions in a way that promotes TMD2-NBD2 to collapse into TMD1-NBD1 in a closed state (red arrows in figure 6D). This would necessitate the exit of any TMD cavity resident lipids that could occur by the extraction of a subset towards the ECD and/or laterally back into the surrounding lipid environment. Apolipoprotein bound ABCA7 could then cycle back and forth between open and closed states in an ATPase hydrolysis dependent manner, repeating the cycle of lipid entry and exit from TMDs in the process. Disengagement of apolipoproteins, possibly in a lipid filled state, would then shift

the conformational equilibrium back to favoring the open state (black arrows in Figure 6D). Given our model, the reduced ATPase stimulation observed for apoE4 compared to apoE3 points to a weaker interaction between apoE4 and ABCA7. Our data therefore offer a direct link between two of the highest genetic risk factors for AD progression, *APOE4* and *ABCA7*, and open up the possibilities for future studies analyzing this direct interaction in context of AD patients carrying mutations in one or both.

Despite significant progress in our understanding of ABCA family transporter structure, several questions about the mechanistic details of their physiological functioning remain open, with several new ones emerging. First, it is yet unclear if ABCA7 and ABCA1 lipid flipping and export properties are connected (Quazi and Molday, 2013; Tomioka et al., 2017). Elucidation of the exact mechanisms involved will shed light on the possible role ABCA7 may play in lipid asymmetry and whether reduced ABCA7 function leads to alterations that may influence cellular phagocytic response. Second, while our data suggests apoE influences ABCA7 activity in an isoform dependent manner, the physiological consequences of this, primarily the reduced effect of the AD related apoE4 mutant, require further study, especially in light of data showing no isoform dependent differences in apoE mediated efflux of lipids in ABCA7 expressing cells (Tomioka et al., 2017). Finally, the underlying molecular basis for lipid specificity in ABCA7 is as of yet poorly understood and requires detailed structural analysis in distinct lipid environments.

METHODS

ABCA7 expression and purification

We utilized the Flp-In TREX system (Thermo Fisher Scientific) for tetracycline inducible expression of human ABCA7. In short, a codon optimized synthetic gene construct (Genart/Thermo Fisher Scientific) of isoform 1 of ABCA7 (Uniprot ID Q8IZY2-1) harboring a

C-terminal eYFP-Rho1D4 tag (Molday and Molday, 2014) with a 3C/precision protease site between the protein and purification tags was cloned into a PCDNA5.1 FRT/TO vector between BamHI and NotI restriction sites and a stable cell line was generated as per manufacturer's protocol. The resulting HEK293 based stable cells were grown and maintained in adherent cell culture in Dulbecco's Modified Eagle Medium (DMEM, Thermo Fisher Scientific) supplemented with 10% Fetal Bovine Serum and a penicillin / streptomycin mixture (Thermo Fisher Scientific) at 37°C with 5% carbon dioxide (CO₂) under humidified conditions. For protein production, cells were induced with 0.6 µg ml⁻¹ tetracycline at a confluency of ~80% in fresh DMEM supplemented with 2% FBS under otherwise identical conditions for an additional 72 hours before being washed with Phosphate Buffered Saline, harvested, and flash frozen in liquid nitrogen.

For purification, thawed cells were resuspended in a lysis buffer (Buffer L) comprising 25 mM Hepes pH 7.5, 150 mM sodium chloride (NaCl), 20% glycerol, and a protease inhibitor cocktail containing 1 cOmplete EDTA free inhibitor tablet (Roche) per 50ml Buffer L, 800 µM phenylmethylsulfonyl fluoride (PMSF), and 10 µg ml⁻¹ soybean trypsin inhibitor (both Sigma), and mechanically cracked using a dounce homogenizer before addition of a 0.5%/0.1% w:v mixture of Dodecyl maltoside (DDM) and Cholesteryl hemisuccinate (CHS) (both Anatrace). Protein extraction was allowed to proceed for 90 minutes at 4°C with gentle agitation after which the suspension was centrifuged at 40,000 rcf for 30 minutes and the supernatant applied to rho-1D4 antibody (University of British Columbia) coupled to cyanogen bromide activated sepharose resin (Cytiva). Binding was allowed to proceed for 3 hours before the unbound fraction was discarded and beads washed with 4 x 10 bed volumes (BVs) of wash buffer (25 mM Hepes pH 7.5, 150 mM NaCl, 0.02%/0.004 % w:v DDM/CHS). Protein was eluted by incubation with 3 BVs

elution buffer (wash buffer supplemented with either 3C protease (1:10 w:w 3C:ABCA7) or 0.5 mg ml⁻¹ 1D4 peptide (genscript)) for 2-18 hours.

apoA1 production and pull-down assay

A synthetic construct of apoA1 bearing a 3C protease cleavable N-terminal decahistidine tag (Genart/Thermo Fisher Scientific) was cloned into a pET28a vector (addgene) and transformed in *E. coli* BL21 DE3 cells (New England Biolabs). One-liter cultures of Terrific Broth (TB) supplemented with 50 ug ml⁻¹ kanamycin were grown from 10 ml overnight cultures from single colonies grown in LB. Cells were grown to an OD600 of 0.8 in a shaking incubator at 37 °C and induced with 1 mM isopropyl β-d-1-thiogalactopyranoside (IPTG). Protein expression was allowed to proceed at 20°C for 12 hours. Cells were centrifuged at 12,000 rcf, and pellets were flash frozen in liquid nitrogen and stored at -80°C until required. Frozen pellets were resuspended in 8 ml/ gram cell pellet resuspension buffer comprising 25 mM Hepes pH 7.5, 150 mM NaCl and 1 mM phenylmethylsulfonyl fluoride (PMSF) and sonicated. The suspension was spun down at 16,000 rcf at 4 °C for 30 min and the supernatant was applied 5 ml Ni-NTA resin (Qiagen) / L culture medium. After discarding the flowthrough, the resin was washed with 25 mM Hepes pH 7.5, 150 mM NaCl, 1 mM PMSF and 20 mM imidazole until a pre-established baseline A280 reading was achieved. ApoA1 was eluted in 4 BVs of 25 mM Hepes pH 7.5, 150 mM NaCl, 1 mM PMSF and 200 mM imidazole, concentrated using a 10 kDa molecular weight cutoff (MWCO) amicon filter (Millipore-Sigma) and desalted using a PD10 column (cytiva) into 25 mM Hepes pH 7.5, 150 mM NaCl. The concentration of apoA1 was adjusted to 1 mg ml⁻¹ for flash freezing in liquid nitrogen and storage at -80°C.

For the pulldown assay, 132 μg of 3C protease cleaved ABCA7 was mixed with pure his tagged apoA1 using a 1:10 molar ratio in a buffer comprising 25 mM Hepes pH 7.5, 150 mM NaCl,

0.02%/0.004 % w:v DDM/CHS and incubated for 2 hours at 4°C with gentle agitation. The mixture was then applied to 100 µl Ni-NTA beads equilibrated with wash buffer, and the binding was allowed to proceed for 2 hours at 4°C with gentle agitation. The beads were washed with 4 x 10 BVs of wash buffer to remove unbound proteins. Bound proteins were eluted with 2 BVs elution buffer (25 mM Hepes pH 7.5, 150 mM NaCl, 0.02%/0.004 % w:v DDM/CHS, 250 mM Imidazole). The eluate was concentrated to 100 µl and the fractions run on sodium dodecyl sulfate (SDS)–polyacrylamide gel electrophoresis (SDS–PAGE).

ABCA7 Nanodisc and proteoliposome preparation

For nanodisc reconstitution, peptide eluted or 3C cleaved ABCA7 was mixed with membrane scaffold protein D1 (MSP1D1, addgene) and a mixture of BPL (Avanti polar lipids) and cholesterol (80:20 w:w) containing 0.5%/0.1% DDM:CHS using a 1:10:350 (ABCA7:MSPD1:lipid mix) molar ratio, in nanodisc buffer (150 mM NaCl, 25 mM Hepes pH 7.5) containing up to 4% glycerol for 30 minutes at room temperature (RT). Nanodisc reconstitution was induced by removing detergent with 0.8 mg⁻¹ml⁻¹ pre-washed Biobeads SM-2 (Bio-rad) for 2 hours with gentle agitation at RT. For different phospholipid compositions, BPL was replaced with brain PE, brain PS, or Brain PS (all from Avanti polar lipids). For structural studies, peptide eluted ABCA7 still bearing the eYFP-Rho1D4 tag was bound to rho-1D4 resin for an additional 2 hours, washed with 4 BV of nanodisc buffer, and eluted with 3C protease for 2 hours at 4°C. The eluted ABCA7 nanodiscs were concentrated using a 100,000 MWCO kDa Amicon filter and further purified by size exclusion chromatography using a G4000swxl column (TOSOH biosciences) equilibrated with nanodisc buffer at 4°C (Figure S1A).

ABCA7 proteoliposomes were generated by mixing detergent purified ABCA7 with a 4:1 mixture of BPL/cholesterol at a protein:lipid ratio of 1:10 w:w using the described protocol (Geertsma et

al., 2008). Proteoliposomes were resuspended at a final concentration of 0.5 - 1 mg ml⁻¹, aliquoted, and flash frozen in liquid nitrogen for storage at -80°C.

ATPase assays

ATPase assays were based on a molybdate based colorimetric assay (Chifflet et al., 1988). Protein concentrations used were in the range of 0.05-0.2 mg ml⁻¹. Assays were started by the addition of 2 mM ATP in the presence of 10 mM magnesium chloride (MgCl₂), incubated for 30 minutes at 37°C, then stopped by addition of 6% SDS. The assay was also performed in the presence of ABCA7 inhibitors as additives, such as 5 mM ATPγS or sodium orthovanadate. For ATP K_M measurements, a range of ATP concentrations was used. Linear regression analyses were carried out using GraphPad Prism. All commercial apolipoproteins were purchased from Abcam, resuspended as per manufacturer specifications and desalted into nanodisc buffer. 6,7-Dimethoxy-2,4-quinazolinediamine (CAS 60547-96-8) was purchased from Toronto Research Chemicals and resuspended in 100% DMSO. All reaction components were mixed with ABCA7 in detergent or reconstituted in nanodiscs in the absence of ATP, incubated for 10 minutes at 37 °C prior to addition of ATP to start the reaction.

Cryo-electron microscopy grid preparation

SEC purified nanodisc reconstituted ABCA7 was mixed with 5 mM ATPγS (Sigma) and 5 mM MgCl₂ for 20 minutes at room temperature and concentrated to 0.2-0.5 mg ml⁻¹. 4 ul samples were applied to glow discharged Quantifoil R1.2/1.3 grids (Electron Microscopy Sciences, Hatfield, PA, USA) using a Vitrobot Mark IV (Thermo Fisher Scientific) with a 4s blotting time and 0 blotting force under >90% humidity at 4°C, then plunge frozen in liquid ethane.

Cryo-electron microscopy data collection and processing.

Grids were clipped as per manufacturer guidelines and cryo-EM data was collected using a Titan Krios electron microscope operating at 300kV and equipped with a Falcon 3EC direct electron detector (Thermo Fisher Scientific.). Automated data collection was carried out using EPU (Thermo Fisher Scientific) over multiple sessions in counting mode at a nominal magnification of 96,000x, corresponding to a calibrated pixel size of 0.895 Å. Image stacks comprising 60 frames were collected at a defocus range of -0.6 to -2.6 µm and estimated dose rate of 1 electron/ Å²/frame and further processed in Relion-3.1 (beta). Motion correction was done using Motioncor2 (Relion implementation)(Zheng et al., 2017) and contrast transfer function (CTF) correction was performed using Gctf (Zhang, 2016). A summary of the overall data processing scheme is presented in Supplementary Figure S1C-E. In brief, 11802 micrographs were used for template free picking of 6725108 particles, followed by particle extraction at a 3x binned pixel size of 2.685 Å/pix. The dataset was processed in two batches. After 2-3 rounds of 2D classification 1259324 particles from Set 1 and 1088487 particles from Set 2 were selected for independent 3D classification steps (number of classes (K)=8 for both). The structure of human ABCA1 (EMDB6724) was used as a 3D reference for an initial 3D classification of a subset of the total data to yield an initial sub-nanometer resolution map of ABCA7 that was used as a 3D reference for the full datasets. After 1 round of 3D classification, both sets of data yielded a similar ensemble of classes. A total of 113291 particles from similar looking classes (black boxes) were subjected to an additional round of classification (K=3), ~80% of which fell into a high-resolution class that yielded a 3.6 Å map after refinement and particle polishing steps. Similarly, 124114 particles from a second set of two similar classes (red boxes in Figure S1D) were selected for subsequent refinement, particle polishing, and post processing to yield a 3.2 Å map. All resolution estimates were based on the gold standard 0.143 cutoff criterion (Scheres and Chen, 2012).

Model building and refinement

Model building was done in coot (Emsley et al., 2010) using a combination of Map 1 and its local resolution filtered variant and Map 2. Both Map 1 and Map 2 displayed significant conformational heterogeneity in the second half of ABCA7, with the quality of density in Map 1 allowing placement of a TMD2 model guided in part by the homologous ABCA1 structure. Density attributed to inter-TMD phospholipids was clearest in Map 1. Map 2 revealed very poor and discontinuous density for TMD2-NBD2 but significantly better density for TMD1-NBD1 and the majority of ECD1 and ECD2, allowing for *de novo* model building. The model for ECD was also guided by the presence of nine glycosylation sites (N78, N98, N312, N340, N1335, N1381, N1386, N1457, & N1518) as well as 4 disulfide bond pairs. Density for the lid region of the ECD was missing in both maps. Model building for both NBDs was guided by structures of the homologous transporters TM287 (Hohl et al., 2012), ABCG2, and ABCA1, where density features did not allow for *de novo* model building. We observed extra density at the nucleotide binding sites for both NBDs despite their open conformation. The structure of the RD was based on a homology model of the predicted RD structure in ABCA1. The quality of density for the ABCA7 RD allowed rigid body placement for the entire domain. Restrained real space refinement of the model was carried out in Phenix (Adams et al., 2010) using automatically generated secondary structure restraints. Structure figures were prepared in UCSF Chimera (Pettersen et al., 2004) and Pymol (The PyMOL Molecular Graphics System, Version 1.8 Schrödinger, LLC).

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Author Contributions

AA conceived the research. LTML, JRT, and AA performed all research. TA and TK provided information on DADMQ and participated in assay design. LTML, JRT, and AA wrote the manuscript with input from all other authors.

Declaration of Interests

The authors declare no competing interests.

Data and materials availability:

The cryo-EM Maps for nanodisc reconstituted human ABCA7 have been deposited at the Electron Microscopy Databank (EMDB) under accession codes EMD-22996 and EMD-22998 for Map 1 and Map 2, respectively. The associated atomic coordinates have been deposited at the Protein Data bank (PDB) under accession code 7KQC.

References

- Abe-Dohmae, S.Y., S. (2012). ABCA7: a potential mediator between cholesterol homeostasis and the host defense system. *Clinical Lipidology* 7, 677-687.
- Adams, P.D., Afonine, P.V., Bunkoczi, G., Chen, V.B., Davis, I.W., Echols, N., Headd, J.J., Hung, L.W., Kapral, G.J., Grosse-Kunstleve, R.W., *et al.* (2010). PHENIX: a comprehensive Python-based system for macromolecular structure solution. *Acta Crystallogr D Biol Crystallogr* 66, 213-221.

474 Alam, A., Kung, R., Kowal, J., McLeod, R.A., Tremp, N., Broude, E.V., Roninson, I.B.,
475 Stahlberg, H., and Locher, K.P. (2018). Structure of a zosuquidar and UIC2-bound human-
476 mouse chimeric ABCB1. *Proc Natl Acad Sci U S A* 115, E1973-E1982.

477 Almeida, J.F.F., Dos Santos, L.R., Trancozo, M., and de Paula, F. (2018). Updated Meta-
478 Analysis of BIN1, CR1, MS4A6A, CLU, and ABCA7 Variants in Alzheimer's Disease. *J Mol*
479 *Neurosci* 64, 471-477.

480 Association, A.s. (2020). 2020 Alzheimer's disease facts and figures. *Alzheimers Dement.*

481 Bellenguez, C., Charbonnier, C., Grenier-Boley, B., Quenez, O., Le Guennec, K., Nicolas, G.,
482 Chauhan, G., Wallon, D., Rousseau, S., Richard, A.C., *et al.* (2017). Contribution to Alzheimer's
483 disease risk of rare variants in TREM2, SORL1, and ABCA7 in 1779 cases and 1273 controls.
484 *Neurobiol Aging* 59, 220 e221-220 e229.

485 Chifflet, S., Torriglia, A., Chiesa, R., and Tolosa, S. (1988). A method for the determination of
486 inorganic phosphate in the presence of labile organic phosphate and high concentrations of
487 protein: application to lens ATPases. *Anal Biochem* 168, 1-4.

488 De Roeck, A., Van Broeckhoven, C., and Sleegers, K. (2019). The role of ABCA7 in
489 Alzheimer's disease: evidence from genomics, transcriptomics and methylomics. *Acta*
490 *Neuropathol* 138, 201-220.

491 Emsley, P., Lohkamp, B., Scott, W.G., and Cowtan, K. (2010). Features and development of
492 Coot. *Acta Crystallogr D Biol Crystallogr* 66, 486-501.

493 Fitzgerald, M.L., Morris, A.L., Rhee, J.S., Andersson, L.P., Mendez, A.J., and Freeman, M.W.
494 (2002). Naturally occurring mutations in the largest extracellular loops of ABCA1 can disrupt its
495 direct interaction with apolipoprotein A-I. *J Biol Chem* 277, 33178-33187.

496 Fitzgerald, M.L., Okuhira, K., Short, G.F., 3rd, Manning, J.J., Bell, S.A., and Freeman, M.W.
 497 (2004). ATP-binding cassette transporter A1 contains a novel C-terminal VLVNFA motif that is
 498 required for its cholesterol efflux and ApoA-I binding activities. *J Biol Chem* 279, 48477-48485.
 499 Geertsma, E.R., Nik Mahmood, N.A., Schuurman-Wolters, G.K., and Poolman, B. (2008).
 500 Membrane reconstitution of ABC transporters and assays of translocator function. *Nat Protoc* 3,
 501 256-266.
 502 Hohl, M., Briand, C., Grutter, M.G., and Seeger, M.A. (2012). Crystal structure of a
 503 heterodimeric ABC transporter in its inward-facing conformation. *Nat Struct Mol Biol* 19, 395-
 504 402.
 505 Hollingworth, P., Harold, D., Sims, R., Gerrish, A., Lambert, J.C., Carrasquillo, M.M., Abraham,
 506 R., Hamshere, M.L., Pahwa, J.S., Moskvina, V., *et al.* (2011). Common variants at ABCA7,
 507 MS4A6A/MS4A4E, EPHA1, CD33 and CD2AP are associated with Alzheimer's disease. *Nat*
 508 *Genet* 43, 429-435.
 509 Ikeda, Y., Abe-Dohmae, S., Munchira, Y., Aoki, R., Kawamoto, S., Furuya, A., Shitara, K.,
 510 Amachi, T., Kioka, N., Matsuo, M., *et al.* (2003). Posttranscriptional regulation of human
 511 ABCA7 and its function for the apoA-I-dependent lipid release. *Biochem Biophys Res Commun*
 512 311, 313-318.
 513 Iwamoto, N., Abe-Dohmae, S., Sato, R., and Yokoyama, S. (2006). ABCA7 expression is
 514 regulated by cellular cholesterol through the SREBP2 pathway and associated with phagocytosis.
 515 *J Lipid Res* 47, 1915-1927.
 516 Lambert, J.C., Ibrahim-Verbaas, C.A., Harold, D., Naj, A.C., Sims, R., Bellenguez, C.,
 517 DeStafano, A.L., Bis, J.C., Beecham, G.W., Grenier-Boley, B., *et al.* (2013). Meta-analysis of

518 74,046 individuals identifies 11 new susceptibility loci for Alzheimer's disease. *Nat Genet* 45,
519 1452-1458.

520 Le Guennec, K., Nicolas, G., Quenez, O., Charbonnier, C., Wallon, D., Bellenguez, C., Grenier-
521 Boley, B., Rousseau, S., Richard, A.C., Rovelet-Lecrux, A., *et al.* (2016). ABCA7 rare variants
522 and Alzheimer disease risk. *Neurology* 86, 2134-2137.

523 Lee, J.Y., Kinch, L.N., Borek, D.M., Wang, J., Wang, J., Urbatsch, I.L., Xie, X.S., Grishin, N.V.,
524 Cohen, J.C., Otwinowski, Z., *et al.* (2016). Crystal structure of the human sterol transporter
525 ABCG5/ABCG8. *Nature* 533, 561-564.

526 Lee, J.Y., Yang, J.G., Zhitnitsky, D., Lewinson, O., and Rees, D.C. (2014). Structural basis for
527 heavy metal detoxification by an Atm1-type ABC exporter. *Science* 343, 1133-1136.

528 Liu, F., Lee, J., and Chen, J. (2021). Molecular structures of the eukaryotic retinal importer
529 ABCA4. *Elife* 10.

530 Ma, F.C., Wang, H.F., Cao, X.P., Tan, C.C., Tan, L., and Yu, J.T. (2018). Meta-Analysis of the
531 Association between Variants in ABCA7 and Alzheimer's Disease. *J Alzheimers Dis* 63, 1261-
532 1267.

533 Manolaridis, I., Jackson, S.M., Taylor, N.M.I., Kowal, J., Stahlberg, H., and Locher, K.P. (2018).
534 Cryo-EM structures of a human ABCG2 mutant trapped in ATP-bound and substrate-bound
535 states. *Nature* 563, 426-430.

536 Meurs, I., Calpe-Berdiel, L., Habets, K.L., Zhao, Y., Korpelaar, S.J., Mommaas, A.M., Josselin,
537 E., Hildebrand, R.B., Ye, D., Out, R., *et al.* (2012). Effects of deletion of macrophage ABCA7
538 on lipid metabolism and the development of atherosclerosis in the presence and absence of
539 ABCA1. *PLoS One* 7, e30984.

540 Molday, L.L., and Molday, R.S. (2014). 1D4: a versatile epitope tag for the purification and
541 characterization of expressed membrane and soluble proteins. *Methods Mol Biol* 1177, 1-15.

542 Nagao, K., Takahashi, K., Azuma, Y., Takada, M., Kimura, Y., Matsuo, M., Kioka, N., and
543 Ueda, K. (2012). ATP hydrolysis-dependent conformational changes in the extracellular domain
544 of ABCA1 are associated with apoA-I binding. *J Lipid Res* 53, 126-136.

545 Nosol, K., Romane, K., Irobalieva, R.N., Alam, A., Kowal, J., Fujita, N., and Locher, K.P.
546 (2020). Cryo-EM structures reveal distinct mechanisms of inhibition of the human multidrug
547 transporter ABCB1. *Proc Natl Acad Sci U S A* 117, 26245-26253.

548 Olsen, J.A., Alam, A., Kowal, J., Stieger, B., and Locher, K.P. (2020). Structure of the human
549 lipid exporter ABCB4 in a lipid environment. *Nat Struct Mol Biol* 27, 62-70.

550 Orlando, B.J., and Liao, M. (2020). ABCG2 transports anticancer drugs via a closed-to-open
551 switch. *Nat Commun* 11, 2264.

552 Pettersen, E.F., Goddard, T.D., Huang, C.C., Couch, G.S., Greenblatt, D.M., Meng, E.C., and
553 Ferrin, T.E. (2004). UCSF Chimera--a visualization system for exploratory research and
554 analysis. *J Comput Chem* 25, 1605-1612.

555 Qian, H., Zhao, X., Cao, P., Lei, J., Yan, N., and Gong, X. (2017). Structure of the Human Lipid
556 Exporter ABCA1. *Cell* 169, 1228-1239 e1210.

557 Quazi, F., and Molday, R.S. (2013). Differential phospholipid substrates and directional transport
558 by ATP-binding cassette proteins ABCA1, ABCA7, and ABCA4 and disease-causing mutants. *J*
559 *Biol Chem* 288, 34414-34426.

560 Scheres, S.H., and Chen, S. (2012). Prevention of overfitting in cryo-EM structure determination.
561 *Nat Methods* 9, 853-854.

562 Sleegers, K. (2020). Expression of ABCA7 in Alzheimer's disease. *Acta Neuropathol* 139, 941-
563 942.

564 Swerdlow, R.H. (2016). Rare ABCA7 variants in Alzheimer disease: Guilt by association.
565 *Neurology* 86, 2118-2119.

566 Tanaka, N., Abe-Dohmae, S., Iwamoto, N., Fitzgerald, M.L., and Yokoyama, S. (2010). Helical
567 apolipoproteins of high-density lipoprotein enhance phagocytosis by stabilizing ATP-binding
568 cassette transporter A7. *J Lipid Res* 51, 2591-2599.

569 Thomas, C., Aller, S.G., Beis, K., Carpenter, E.P., Chang, G., Chen, L., Dassa, E., Dean, M.,
570 Duong Van Hoa, F., Ekiert, D., *et al.* (2020). Structural and functional diversity calls for a new
571 classification of ABC transporters. *FEBS Lett* 594, 3767-3775.

572 Tomioka, M., Toda, Y., Manucat, N.B., Akatsu, H., Fukumoto, M., Kono, N., Arai, H., Kioka,
573 N., and Ueda, K. (2017). Lysophosphatidylcholine export by human ABCA7. *Biochim Biophys*
574 *Acta Mol Cell Biol Lipids* 1862, 658-665.

575 Voloshyna, I., and Reiss, A.B. (2011). The ABC transporters in lipid flux and atherosclerosis.
576 *Prog Lipid Res* 50, 213-224.

577 Wang, N., Lan, D., Gerbod-Giannone, M., Linsel-Nitschke, P., Jehle, A.W., Chen, W., Martinez,
578 L.O., and Tall, A.R. (2003). ATP-binding cassette transporter A7 (ABCA7) binds apolipoprotein
579 A-I and mediates cellular phospholipid but not cholesterol efflux. *J Biol Chem* 278, 42906-
580 42912.

581 Wu, Y.C., and Horvitz, H.R. (1998). The *C. elegans* cell corpse engulfment gene *ced-7* encodes a
582 protein similar to ABC transporters. *Cell* 93, 951-960.

583 Zhang, K. (2016). Gctf: Real-time CTF determination and correction. *J Struct Biol* 193, 1-12.

Zheng, S.Q., Palovcak, E., Armache, J.P., Verba, K.A., Cheng, Y., and Agard, D.A. (2017).
MotionCor2: anisotropic correction of beam-induced motion for improved cryo-electron
microscopy. *Nat Methods* 14, 331-332.

Figures and Legends

Figure 1. Overall structural and functional characterization of human ABCA7 (A) ABCA7 ATPase activity in nanodiscs of different phospholipid/cholesterol compositions. (B) ATPase. Activity of ABCA7 at different ATP concentrations. (C) ABCA7 ATPase rates in the presence of apoA1 and apoE1-4. (D) SDS-PAGE gel of ABCA7-apoA1 pulldown. L= ABCA7 + apoA1 Load. FT= flowthrough, W=final wash, E = eluate, C= concentrated eluate, M= molecular weight marker. (E) Overall structure of nanodisc reconstituted ABCA7. The two halves are shown as yellow and orange ribbons, respectively. Bound nucleotides are shown as solid spheres. Grey bars represent the membrane upper leaflet (UL) and lower leaflet (LL) and modeled lipids as transparent

green spheres. **(F)** Local resolution filtered cryo-EM map of human ABCA7. Dashed red ovals demarcate location of TMD lipids. ATPase data reported are for three independent experimental replicates (n=3) and error bars represent standard.

Fig. 2. Details of TMD and lipid filled transmembrane pathway (A) Central lipid filled transmembrane cavity with cavity lining TMs shown as ribbons. Intracellular gate forming residues L655 and T1646 are shown as yellow and orange spheres, respectively. Green sticks indicate lipids and EM density is shown as blue mesh contoured at 6σ . (B) same as panel A viewed from side (C) Detailed view from panel B with sidechains from residues within 5 Å of observed TMD lipids shown as sticks and labeled. Lipids are represented as transparent green spheres.

Fig. 3. Details of ECD-TMD and lipid facing interfaces. Electrostatic potential maps focusing on (A) TMD1, (B) TMD2, and (C) the ECD. Lipids are shown as transparent green spheres, TMD1 in yellow (B-C), TMD2 in orange ribbon (A, C) the ECD in cyan (A, B) colored ribbons. **D** TMD-ECD binding interface with TMD1 and TMD2 colored yellow and orange respectively, and ECD1 and ECD 2 colored purple and cyan, respectively. TMD1 and TMD2 residues within 4Å of either ECD are labeled yellow and orange, respectively. ECD1 and ECD2 residues within 4Å of either TMD are labeled purple and cyan, respectively. (E) Unspecified ECD lipids (magenta sticks) and their corresponding EM density (blue mesh) within the ECD hydrophobic tunnel. TMD 1 and TMD 2 are colored yellow and orange respectively, molecules are shown as green sticks. Ca atoms for residues within 5Å of observed lipids are shown as spheres.

Figure 4 Known amino acid variants of ABCA7. Structure of ABCA7 with C α atoms shown for pathogenic variants (green), protective variants (red), and residues conserved in ABCA1 and ABCA7 known to disrupt the latter's binding to apoA1 and/or phospholipid translocation (blue). Cyan spheres highlight residue positions of ABCA1 mutations known to disrupt phospholipid efflux including those equivalent to the VFVNFA motif within the ABCA1 RD. Grey bars indicate the upper and lower membrane bilayer leaflets (UL and LL, respectively).

Figure 5. Conservation of ABCA7 and ABCA1 structural elements (A) Ribbon representation of select ABCG family transporter structures with rcsb codes. Overall structural alignment of ABCA7 (blue) and ABCA1 (rose). (B) Alignment of TMD1 and TMD2 of ABCA1 and ABCA7 with individual TMs labeled, viewed from the extracellular side. (C) Same as panel B, viewed from the cytoplasmic side. (D) Front view of NBD1, NBD2, and the RD of ABCA7 aligned to those of ABCA. (E) ABCA7-ABCA1 NBD alignment viewed from the extracellular side with the ECD and TMDs removed for clarity.

Figure 6. Conservation of ABCA and ABCG family structural elements (A) Ribbon representation of select ABCG family transporter structures and their respective PDB IDs including apo open ABCG2 (black), closed ATP bound structure of ABCG2_{EQ} (black), and ABCG5/G8 (cyan and green, respectively) (B) Alignment of NBD-TMD pairs from open and closed conformations of ABCG2 (left), ABCG2 and ABCG5/G8 (center), and closed ABCG2 with TMD-NBD pairs from ABCA7 half 1 (gold) and half 2 (orange). (C) Alignment of both ABCA7 TMD-NBD pairs with NBD-TMD pair from closed ABCG2_{EQ}-ATP (left) and superposition of the full ABCG2_{EQ}-ATP structure onto ABCA7 aligned with respect to TMD1-NBD1. Arrow and question mark indicate proposed transition from the former, open state to the latter hypothetical closed state based on ABCG2 with ECD and ED shown as transparent ribbons. (D) Hypothetical

model of apolipoprotein induced structural transitions in ABCA7. The ECD TMD interface is shown as solid black rectangle or dashed rectangle. Phospholipids are shown as green lines.

Figure S1. Cryo-EM data processing workflow for nanodisc reconstituted ABCA7 (A) Size exclusion chromatography micrograph of cryo-EM sample showing monodisperse ABCA7 nanodisc (main peak). (B) ATPase data nanodisc reconstituted ABCA7 in the presence of sodium orthovanadate (VO₄), ATPγS, and DADMQ normalized to apo ABCA7 ATPase rate. N=3 and error bars represent s.d. C Representative micrograph at -2.5 μm defocus. Scale bar = 20 nm. D EM processing workflow. Boxes indicate 3D classes used for further refinement for both Map 1 and

701 Map 2 (red). **E** Fourier shell correlation (FSC) curves for Map 1 (top) and Map 2 (bottom). Dotted
702 lines indicate position 0.143 and 0.5 cutoff criteria for resolution estimates.

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Figure S2. Local resolution-colored maps Central slices through Map 1 (top) and Map 2 (bottom)

colored by local resolution.

Figure S3. Quality of EM density for ABCA7 nanodisc TM helices are numbered. ECD density is shown for Map 2, all other density is for Map 1 contoured at 6σ . TMD phospholipids (modeled as phosphatidylcholine molecules) are shown as green sticks, Glycans as black sticks, and ATP γ S (AGS) molecules as red sticks. EH = external helix. UL and LL represent upper leaflet and lower leaflet lipids, respectively.

Figure S4. ABCA7 and ABCA4 alignment Superpositions of individual domains of nanodisc reconstituted ABCA7 and detergent purified ABCA1. Individual TMD-NBD pairs were separately aligned (Half 1 and Half 2 for TMD1-NBD1 and TMD2-NBD2, respectively) as were the ECD and RD.

Figure S5 Sequence alignment of human ABCA7 and human ABCA1 Blue dots indicate residues known to be important for apoA1 binding in ABCA1. Numbers indicate disulfide bond pairs. Green and red dots indicate residue positions of naturally occurring pathogenic or protective

variants in ABCA7, respectively. Cyan dots indicate residue positions in ABCA1 thought to disrupt phospholipid efflux.

Dataset	Nanodisc reconstituted human ABCA7	
Magnification	96k	
Pixel Size (Å)	0.895	
Total Dose (e/Å ²)	60	
Defocus Range (um)	-0.8 to 2.6	
Maps	Map 1	Map 2
EMDB ID	EMD-22996	EMD-22998
# Particles in final Class	91381	124114
Resolution (Å) (0.143 threshold)	3.6	3.2
Sharpening B factor		
Refined Coordinates	Human ABCA7	
PDB ID	7KQC	
# Residues/Non-hydrogen Atoms/Ligands	1801/14611/28	
R.M.S deviations		
Bond Angles (Å)	0.006	
Bond lengths (°)	0.824	
MolProbity Statistics		
Molprobity Score	1.84	
Clashscore	8.84	
Poor rotamers (%)	0.41	
Ramachandran statistics		
Favored (%)	94.72	

Allowed (%)	4.83
Outliers (%)	0.45

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781 **Table S1 Data collection and refinement statistics.**

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