THE EMBO JOURNAL

Cryo-EM Structures of human ABCA7 provide insights into its phospholipid translocation mechanisms

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Transaction Report:

(Note: With the exception of the correction of typographical or spelling errors that could be a source of ambiguity, letters and reports are not edited. Depending on transfer agreements, referee reports obtained elsewhere may or may not be included in this compilation. Referee reports are anonymous unless the Referee chooses to sign their reports.)

Dear Dr Alam,

Thank you again for the submission of your manuscript entitled "Capturing the interplay of membrane lipids and structural transitions in human ABCA7". I have now received the referees' reports, which are copied to the bottom of this message.

At its heart, all referees agree that the work is based on a technically accomplished and well-described collection of protein structures. They also state unambiguously that the manuscript is timely and the topic is important. However, the feedback was not unambiguously positive. The data that you present will need to be given a wider mechanistic context if they are to be published in EMBO Journal.

I would like to invite you to address the comments of all referees in a revised version of the manuscript. In particular, more experiments and a refined round of modelling will be needed to advance our understanding of ABCA7 transport mechanics, as outlined by the reviewers. If you judge that such experiments are not feasible within a reasonable time-frame, it may be in your best interests to submit the study elsewhere. Our usual revision time of three months is used as a guideline, not a deadline; manuscripts frequently take longer to revise. I will be available and happy to talk next week if you have any questions, I recommend that we go over our next steps and discuss the referees' comments further over Zoom.

I should add that it is The EMBO Journal policy to allow only a single major round of revision and that it is therefore important to resolve these concerns at this stage. I believe the concerns of the referees are reasonable and addressable, but please contact me if you have any questions, need further input on the referee comments or if you anticipate any problems in addressing any of their points. Please, follow the instructions below when preparing your manuscript for resubmission.

I would also like to point out that as a matter of policy, competing manuscripts published during this period will not be taken into consideration in our assessment of the novelty presented by your study ("scooping" protection). We have extended this 'scooping protection policy' beyond the usual 3 month revision timeline to cover the period required for a full revision to address the essential experimental issues. Please contact me if you see a paper with related content published elsewhere to discuss the appropriate course of action.

When preparing your letter of response to the referees' comments, please bear in mind that this will form part of the Review Process File, and will therefore be available online to the community. For more details on our Transparent Editorial Process, please visit our website: https://www.embopress.org/page/journal/14602075/authorguide#transparentprocess

Again, please contact me at any time during revision if you need any help or have further questions.

Thank you very much again for the opportunity to consider your work for publication. I look forward to your revision.

Best regards,

William Teale

William Teale, Ph.D.
Editor
The EMBO Journal

When submitting your revised manuscript, please carefully review the instructions below and include the following items:

1) a .docx formatted version of the manuscript text (including legends for main figures, EV figures and tables). Please make sure that the changes are highlighted to be clearly visible.

2) individual production quality figure files as .eps, .tif, .jpg (one file per figure).

3) a .docx formatted letter INCLUDING the reviewers' reports and your detailed point-by-point response to their comments. As part of the EMBO Press transparent editorial process, the point-by-point response is part of the Review Process File (RPF), which will be published alongside your paper.

4) a complete author checklist, which you can download from our author guidelines (https://wol-prod-cdn.literatumonline.com/pb-assets/embo-site/Author Checklist%20-%20EMBO%20J-1561436015657.xlsx). Please insert information in the checklist that is also reflected in the manuscript. The completed author checklist will also be part of the RPF.

5) Please note that all corresponding authors are required to supply an ORCID ID for their name upon submission of a revised

manuscript.

6) We require a 'Data Availability' section after the Materials and Methods. Before submitting your revision, primary datasets produced in this study need to be deposited in an appropriate public database, and the accession numbers and database listed under 'Data Availability'. Please remember to provide a reviewer password if the datasets are not yet public (see https://www.embopress.org/page/journal/14602075/authorguide#datadeposition). If no data deposition in external databases is needed for this paper, please then state in this section: This study includes no data deposited in external repositories. Note that the Data Availability Section is restricted to new primary data that are part of this study.

Note - All links should resolve to a page where the data can be accessed.

7) When assembling figures, please refer to our figure preparation guideline in order to ensure proper formatting and readability in print as well as on screen:
http://bit.ly/EMBOPressFigurePreparationGuideline

Please remember: Digital image enhancement is acceptable practice, as long as it accurately represents the original data and conforms to community standards. If a figure has been subjected to significant electronic manipulation, this must be noted in the figure legend or in the 'Materials and Methods' section. The editors reserve the right to request original versions of figures and the original images that were used to assemble the figure.

8) For data quantification: please specify the name of the statistical test used to generate error bars and P values, the number (n) of independent experiments (specify technical or biological replicates) underlying each data point and the test used to calculate p-values in each figure legend. The figure legends should contain a basic description of n, P and the test applied. Graphs must include a description of the bars and the error bars (s.d., s.e.m.).

9) We would also encourage you to include the source data for figure panels that show essential data. Numerical data can be provided as individual .xls or .csv files (including a tab describing the data). For 'blots' or microscopy, uncropped images should be submitted (using a zip archive or a single pdf per main figure if multiple images need to be supplied for one panel). Additional information on source data and instruction on how to label the files are available at .

10) We replaced Supplementary Information with Expanded View (EV) Figures and Tables that are collapsible/expandable online (see examples in https://www.embopress.org/doi/10.15252/embj.201695874). A maximum of 5 EV Figures can be typeset. EV Figures should be cited as 'Figure EV1, Figure EV2" etc. in the text and their respective legends should be included in the main text after the legends of regular figures.

- For the figures that you do NOT wish to display as Expanded View figures, they should be bundled together with their legends in a single PDF file called *Appendix*, which should start with a short Table of Content. Appendix figures should be referred to in the main text as: "Appendix Figure S1, Appendix Figure S2" etc. See detailed instructions regarding expanded view here: .

- Additional Tables/Datasets should be labelled and referred to as Table EV1, Dataset EV1, etc. Legends have to be provided in a separate tab in case of .xls files. Alternatively, the legend can be supplied as a separate text file (README) and zipped together with the Table/Dataset file.

11) Our journal encourages inclusion of *data citations in the reference list* to directly cite datasets that were re-used and obtained from public databases. Data citations in the article text are distinct from normal bibliographical citations and should directly link to the database records from which the data can be accessed. In the main text, data citations are formatted as follows: "Data ref: Smith et al, 2001" or "Data ref: NCBI Sequence Read Archive PRJNA342805, 2017". In the Reference list, data citations must be labeled with "[DATASET]". A data reference must provide the database name, accession number/identifiers and a resolvable link to the landing page from which the data can be accessed at the end of the reference. Further instructions are available at .

Further instructions for preparing your revised manuscript:

Please make sure you upload a letter of response to the referees' comments together with the revised manuscript.

Please also check that the title and abstract of the manuscript are brief, yet explicit, even to non-specialists.

When assembling figures, please refer to our figure preparation guideline in order to ensure proper formatting and readability in print as well as on screen:
https://bit.ly/EMBOPressFigurePreparationGuideline
See also guidelines for figure legends: https://www.embopress.org/page/journal/14602075/authorguide#figureformat

IMPORTANT: When you send the revision we will require
- a point-by-point response to the referees' comments, with a detailed description of the changes made (as a word file).
- a word file of the manuscript text.
- individual production quality figure files (one file per figure)
- a complete author checklist, which you can download from our author guidelines
(https://www.embopress.org/page/journal/14602075/authorguide).
- Expanded View files (replacing Supplementary Information)
Please see out instructions to authors
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Further information is available in our Guide For Authors: https://www.embopress.org/page/journal/14602075/authorguide

We realize that it is difficult to revise to a specific deadline. In the interest of protecting the conceptual advance provided by the work, we recommend a revision within 3 months (11th Jul 2022). Please discuss the revision progress ahead of this time with the editor if you require more time to complete the revisions. Use the link below to submit your revision:

https://emboj.msubmit.net/cgi-bin/main.plex

Referee #1:

This paper presented by Le et al. reports on the structural elucidation of the human ABC transporter ABCA7 by cryo-EM. The core of the paper are four structures of this member of the ABCA sub-family, three determined in the absence of nucleotides or in the presence of ATP-gamma-S (which in fact does not close the nucleotide binding domains (NBDs)) and the other one carrying the Walker B EtoQ mutation in the NBDs and in the presence of ATP. As a consequence of nucleotide trapping at the NBDs of the EtoQ mutant, the transmembrane domains (TMDs) adopt a closed state (when the NBD pair sandwiches two ATP molecules); in the absence of nucleotides or in the presence of ATP-gamma-S, the TMDs adopt an open conformation. The ABCA7 structure with open TMDs was solved in nanodiscs either using brain total lipids + cholesterol or the defined PE lipids or using the detergent digitonin. This allowed a direct comparison of the conformations in nanodiscs and detergent. While this is not the first structure of a member of the ABCA sub-family, it is the first structure for human ABCA7 itself. The overall fold is similar to the one of ABCA1, but also to members of the ABCG subfamily, and can be classified as type II exporter (or type V ABC transporter). A special feature of the nucleotide-free ABCA7 structures determined in nanodiscs is the presence of a relatively large cavity surrounded by the TMDs, which is in fact considerably larger than the ones previously observed for other members of the ABCA sub-family, and interestingly contains density that can be clearly interpreted as a small patch of a lipid bilayer. In detergent, the TMD cavity is considerably smaller and contains some loosely bound detergent molecules (or lipids, hard to say). Molecular dynamics simulations finally support a transport mechanism in which binding of the bilayer patch within the TMD cavity results in upward protrusion of the patch of lipids and may aid extraction of lipids to the extracellular domains, which is further supported by NBD/TMD closure.
Overall, the paper is very clearly written and importantly, it places the own results into the larger context of the ABC transporter field by comparing the ABCA7 structures to existing structures and citing the relevant literature. I see mainly one experimental weakness, that is that no experiments with mutants of ABCA7 were performed, which in my view is a missed opportunity. Further, the authors are extremely careful in taking a clear stand on what they think this transporter is exactly doing/transporting and how the data can be interpreted. One reads the paper and asks: well, these are nice structures, but what is the take home lesson? With the bilayer patch and the upward protrusion seen in the simulations, the ingredients for a clearer message are definitely there. I would like to invite the authors to dare to speculate a bit more about their data and come up with a credible mechanism.
Below I provide some ideas on how this nice work can be further strengthened and sharpened.

Major points
1) The chemical nature of lipids transported by ABCA7 seems to be unclear (at least that is what I grasp of the introduction). In line 219ff of the discussion, it is mentioned that ABCA7 may play some role in phosphatidyl-serine (PS) transporter, which indeed is a lipid whose asymmetric distribution plays a key regulatory role in a number of cellular processes. In Figure 1A, ATPase activity of ABCA7 is measured when reconstituted in nanodiscs with different lipds, showing that the protein is maximally active in phosphatidyl-ethanolamine (PE). Although kind of "promised" in the introduction (line 39ff: To date, no direct structural information exists for ABCA7. Understanding the molecular details of the ABCA7 transport cycle and how its

dysfunction alters inflammatory and immune responses, lipid homeostasis, and phagocytosis, which all contribute to AD progression, may therefore pave the way for novel therapeutics for AD.), the paper does not even try to shed further light on the question on what lipids are flipped or extracted by ABCA7. Of course one can argue that with the current resolution of the structure, it is difficult to reach any conclusions, but at least in the discussion, one may dare a bit more speculation. What would of course be great is to learn what kind of annular lipids are "selected" by ABCA7 from the total lipids of the cells in which it is overexpressed and from which it is purified. This could be assessed by mass spectrometry/lipidomics.
2) Figure 1E highlights a number of positively charged residues which appear to interact with the head-groups of the phospholipids. Some of them seem to be mutated in patients suffering from AD (line 209, R475 being mutated in AD patients). I see it as a missed opportunity that the authors did not attempt to mutate 2-3 of these positively charged residues into alanine and performed at least purifications, nanodiscs reconstitution and ATPase activity assays. What would be even nicer are some transport assays with such mutants. In any case, it would help the story if it contained some data going beyond structural descriptions (having said this, the structural descriptions are very well done with a right level of details!).

Minor points
1) Figure 4 is hard to "read". In particular, one does not see the conformational differences very well in the very small overlay in Fig. 4A. The fact that the subdomains move as rigid body is interesting (but not the main point in my view); rather, it is interesting to have captured ABCA7 in three main conformations. I am sure that with a bit of effort one can make this figure look nicer and more informative.
2) Likewise, I feel that Figure 6 (the mechanism figure) is too minimalistic. Such a thing might have been acceptable 20 years ago when we had no clue about ABC transport structure/function. The ABCA sub-family is by now pretty well-studied, with a good number of structures in the "game". Likewise, there are certainly some potential mechanisms that are currently discussed, among which of course the core questions on the whether these ABC transporters flip, flop or extract lipids! This should be somehow made visible. Next to fact that the ECDs likely play a major role.

Referee #2:

Le et al report the single particle cryo-EM structures of ABCA7 in the nucleotide-free and nucleotide bound state at resolutions of 3.6 and 4.0 Å, respectively. In the open, nucleotide-free state, lipids are present corresponding to the position of the inner and outer leaflet of the bilayer. Interestingly, no lipids were detected in the ATP-bound state that corresponds to the slightly outward-open state of ABCB7.

Structures of ABCA1 and ABCA4 have already been described. New in these structures is the presence of a lipid belt in the apo state, which is absent in the outward-open state and allows proposing of a model of lipid transport / flipping of ABCA7.

Overall the manuscript is in principle suitable for EMBO Journal, although a couple of points have to be addressed prior to a final decision.

I listed my points / concerns in order of appearance and not importance:

- Line 63: The authors should provide a Table in SI summarizing KM and vmax values of ABCA7 in different environments. Also, what means drastically reduced ATPase activity for the EQ double mutant? A quick look at Figure S1A seems to tell me that the EQ has some 15% residual activity. This is substantial activity for a catalytic base residue! And higher as in the presence of ATPgammaS. This has to be clarified.
- Figure S1A: I only see one bar for the EQ mutant, but the text reads for me that activity as determined in nanodiscs and detergent.
- Line 75: I would suggest to recommend the statement concerning cholesterol. Data is presented only for nanodiscs (ND). Thus, I would claim an inhibitors effect only for ND or repeat he experiments in other environments.
- Line 95: A rmsd of 0.3 Å is identical and not 'nearly identical' at this resolution.
- Line 102 - 104: What is the driving force of lipids to entry the hydrophilic cavity of ABCA1?
- Line 117 - 118: As expected, ABCA7 in detergent does not show lipids in the cavity. What is the rational to claim that the lipids in ND are specifically bound to the protein and not simply a result of lipid crowding in the ND?
- Line 120 - 121: Lipids in the ND are free to diffuse. In other words, they are less ordered and can be seen only at lower contour level. Again, this is no argument for specificity in my opinion. I would expect to generate mutants of for example the positively charged cluster, which interacts with the lipids. This would be a strong support of specificity, If a chance is observed at all.
- Line 157: Is a translation of 2 Å at this resolution really something one should argue with?
- Line 170: Please provide the rmsd values for the comparison.
- Line 177: Can the MD simulations explain an inhibitory effect of cholesterol? It should bind to the protein.
- Line 202: I wonder whether ABCA7 has a lipid specificity? According to the proposed model, ABCA7 would pump every lipid present in the inner leaflet to the outer leaflet. But this would abolish any lipid gradient such as the well-known PS gradient. Or am I missing something here? The same line of arguments holds for line 216- 217.

- Figure S9 and S10: I think that three sequence are truly not sufficient to make a claim that residues are conserved. More sequence can easily be incorporated and analyzed.
- Line 220: If ABCA7 flips PS (ref 16) again where is the specificity of flipping coming from? And this relation structural findings imposed on biology is my major concern with the manuscript.
- Line 321: if 2 mM ATP was used for the purification of the EQ mutant, one could calculate whether a 15% residual activity of the EQ double mutant would hydrolyze sufficient amounts of ATP as the author state that ATP is bound to the NBD.
- Line 357: What concentration of orthovandate was used? How was it prepared. This is important in light of the efficiency of vanadate trapping.
- Figure S1A: Why is the trapping efficiency of vanadate so poor? Approximately only 1/3 of the activity is lost, but vanadate is known to be an efficient inhibitor of P-loop ATPases!

Referee #3:

The manuscript by Le et al. describes a structural study on ABCA7 combining cryo-EM, ATPase assays and MD simulations. The study revealed the structure of ABCA7 in three conformations. The topic of the study is interesting and could contribute to our understanding of ABC transporter function, importantly to the structurally understudied ABCA family, but unfortunately, the manuscript shows several shortcomings.

Claims are not supported by the data provided in the results section.
- The manuscript starts with a very promising title "Capturing the interplay of membrane lipids and structural transitions in human ABCA7", but it can't maintain the claim. The manuscript shows cryo-EM structure, which are static and it reports MD simulations, in which the protein is fixed and can't move, thus no transitions can be relieved. Author should find a title which better describes the content of manuscript.
- It is not clear how the presented data would have a broader impact on the ABCA family, as the wide separation between TMD1 and TMD2 seems limited to ABCA7.
- In the introduction (line 32-34) the authors make the claim to explain the substrate transport cycle. The results to not show new evidence to support the claim.
- Line 40-43, the claim to understand a large number or diseases is far fetched.
- Line 53-55, the author incorrectly promise to directly visualize conformational changes associated with lipid partitioning. In reality, in the MD studies, the conformation of the protein is fixed by position restraints, thus these changes cannot be investigated with the presented simulations, while in the cryo-EM data, a direct and causality-linked structural response to lipid partitioning has not been shown. Also, line 92-96 the authors state, that the change in lipid composition had no structural effect, which seems to contradict the statement in line 53-55.
- Line 223-224: I can't see the significant progress in our mechanical understanding ... . The structures are new and important, but the progress at the level of transporter mechanics it not become clear from the current manuscript.

ATPase data:
-Information on number of repeats, concentrations, conditions, biochemical and statistical analysis is frequently missing for data shown in Fig1 and FigS1.
-ABCA7 has been proposed to be a PS transporter, much less of PE or PC. Please seen doi.org/10.1074/jbc.M113.508812 as an example. How can Fig1B be reconciled with this information? Could you give any reason, why the ATPase activity of PE is much higher then for PS.
-Vanadate trapping is probably the most widely used treatment to block ATPase activity across the ABC transporter superfamily. In Fig S1A, vanadate treatment reduces ATPase activity by less the 50%. Is this expected? Can this behaviour be explained?

Simulation data:
-Which version of the Martini force field was used?
-For direct comparison between cryo-EM data and simulation data, these should use the same conditions. Currently, the cryo-EM - PE structure is cholesterol free, while the simulation employ a PE:cholesterol mixture.
-I do not fully understand the dome-shape statement. Figure 5C,D and Fig S8A,B suggest this is actually not not the case. At the extracellular leaflet, the highest elevation is at the edge of the TMD-lipid interface and extends as much into the membrane as into the cleft between TMD1-TMD2. Second, this is only visible at one side of the transporter. At the cytoplasmic side, the 2D plot suggest the opposite of a dome, as the centre is further away from the centre of the membrane. This is seen for both PC and PE. Given that the PE lipids create strong membrane curvature, while a PC/Chol membrane is in its biophysical properties closer to and eukaryotic cell membrane, I would suggest to flip the PE and PC dataset between Fig 5 and Fig S8.
-The results from the quantification of phyospholipids is hampered by the fact that the protein Ca atoms remain fixed in the conformation observed in the cryo-EM structure. Therefore, ABCA7 cannot move or dynamically react on interactions of lipids, while lipids might be force to adopt distributions, which are only induced because the geometry of the restrained transporter. At least, after the 2 µs, the simulations need to be extended for sufficient time without restraints.
-The preference of partitioning of PE over PC is uncertain. This is difficult to reconcile with the data shown in Fig 5. Fig 5A/insert, which would not indicate the preference. Also, Fig 5B does not support the statement, because the standard

deviations are strongly overlapping. It would be helpful to show the actual distributions histograms or the time evolution individually for each of the four transporters, as the bar graph is hiding some of important details.

Other

-The idea of an exit pocket is interesting, but has not been explored beyond suggesting its existence and reporting its geometry. The link to the loop containing residues 475, 478, 482 as well as 678 could be better explored, but current simulations are limited by the position restraints.

-Figure 4E suggests a larger intradomain motion of the NBDs of the α-helical domain vs the recA-like domain, which was observed before in several other ABC transports. If true, this could be explored, but would also imply that the statement, that all motions are rigid body motions, is not correct.
-Line 175-176: The fact that ABC transporter show a large contribution of rigid body motions as shown here is not novel, but well established.
-The comparison to the ABCG is interesting, but more important is a comparison to the closely related ABCA1 and ABCA4 structures and conformations, which both have been solved in more than one conformation.

-I do not understand the use of the ABCB family transporter TM287 during the model building process. It has a has a different TMD fold. Please explain. Also, citation 51 is not citing any work on TM287.

-The model of Fig 6 is incomplete for my understanding: The RD region holds the ABCA7 dimer together at the cytosolic site, the ECD at the extracellular side. Also, the model shows a lipid half flipped inside the transporters. Is there any evidence for this speculation from MD or cryo-EM? It is also not described in text, if the authors assume that the substrate lipid would change orientation within the transporter. It could e.g. as well occur in the exit pocket.

-For reporting on lipid transport and interaction with the transporter, simulations will need to include PS lipids, which according to current knowledge, are the most likely substrates.

Minor points:
line 62: It should read "Michaelis-Menten constant" instead of "Michaelis constant".
line 63: Please provide exact numbers for the Km in the different conditions.
line 89: Should it read TMD1-ECD1 or TMD2-ECD1. Please check.
line 99: Is ABCA7BPE correct? Please check.
line 209-211. A deformation of the membrane plane will require energy input. If it is not caused by the position restraints in the first place, this deformation will very likely serve as an energetic and kinetic barrier instead of aiding (accelerating) binding of lipids, as proposed.
Methods: please confirm that HEK293 cells were used for WT ABCA7, while HEK293T cells were used for the mutants.
Figure 5: The colour legend on panel C/D are very difficult to identify. Please use a clearer representation
Figure 6S: Please add residues labels
Figure S8E: it is unclear, which type of protein representation is used.

Referee #1:

This paper presented by Le et al. reports on the structural elucidation of the human ABC transporter ABCA7 by cryo-EM. The core of the paper are four structures of this member of the ABCA sub-family, three determined in the absence of nucleotides or in the presence of ATP-gamma-S (which in fact does not close the nucleotide binding domains (NBDs)) and the other one carrying the Walker B EtoQ mutation in the NBDs and in the presence of ATP. As a consequence of nucleotide trapping at the NBDs of the EtoQ mutant, the transmembrane domains (TMDs) adopt a closed state (when the NBD pair sandwiches two ATP molecules); in the absence of nucleotides or in the presence of ATP-gamma-S, the TMDs adopt an open conformation. The ABCA7 structure with open TMDs was solved in nanodiscs either using brain total lipids + cholesterol or the defined PE lipids or using the detergent digitonin. This allowed a direct comparison of the conformations in nanodiscs and detergent. While this is not the first structure of a member of the ABCA sub-family, it is the first structure for human ABCA7 itself. The overall fold is similar to the one of ABCA1, but also to members of the ABCG subfamily, and can be classified as type II exporter (or type V ABC transporter). A special feature of the nucleotide-free ABCA7 structures determined in nanodiscs is the presence of a relatively large cavity surrounded by the TMDs, which is in fact considerably larger than the ones previously observed for other members of the ABCA sub-family, and interestingly contains density that can be clearly interpreted as a small patch of a lipid bilayer. In detergent, the TMD cavity is considerably smaller and contains some loosely bound detergent molecules (or lipids, hard to say). Molecular dynamics simulations finally support a transport mechanism in which binding of the bilayer patch within the TMD cavity results in upward protrusion of the patch of lipids and may aid extraction of lipids to the extracellular domains, which is further supported by NBD/TMD closure.
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We thank the reviewer for their positive assessment of our work. Below we have done our best to respond to the reviewer's comments.

Below I provide some ideas on how this nice work can be further strengthened and sharpened.

Major points
1) The chemical nature of lipids transported by ABCA7 seems to be unclear (at least that is what I grasp of the introduction). In line 219ff of the discussion, it is mentioned that ABCA7 may play some role in phosphatidyl-serine (PS) transporter, which indeed is a lipid whose asymmetric distribution plays a key regulatory role in a number of cellular processes. In Figure 1A, ATPase

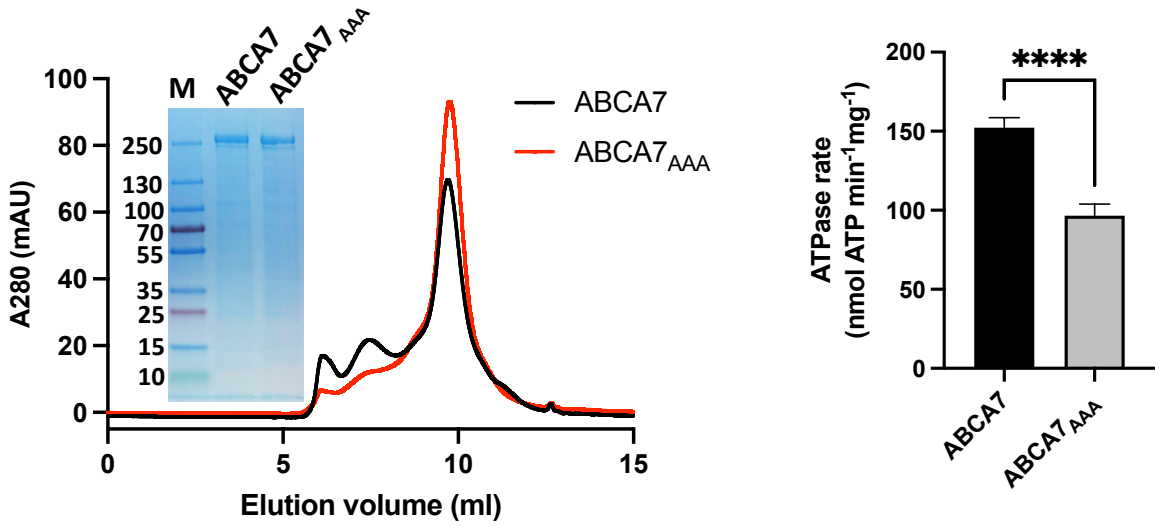
activity of ABCA7 is measured when reconstituted in nanodiscs with different lipds, showing that the protein is maximally active in phosphatidyl-ethanolamine (PE). Although kind of "promised" in the introduction (line 39ff: To date, no direct structural information exists for ABCA7. Understanding the molecular details of the ABCA7 transport cycle and how its dysfunction alters inflammatory and immune responses, lipid homeostasis, and phagocytosis, which all contribute to AD progression, may therefore pave the way for novel therapeutics for AD.), the paper does not even try to shed further light on the question on what lipids are flipped or extracted by ABCA7. Of course one can argue that with the current resolution of the structure, it is difficult to reach any conclusions, but at least in the discussion, one may dare a bit more speculation. What would of course be great is to learn what kind of annular lipids are "selected" by ABCA7 from the total lipids of the cells in which it is overexpressed and from which it is purified. This could be assessed by mass spectrometry/lipidomics.

1.1 As per the reviewer's suggestion, we have added data analyzing annular lipids associated with detergent purified ABCA7 by thin layer chromatography, which qualitatively suggests that PE, PC, and PS copurify with the protein. It is, however, difficult to distinguish which of these lipids are associated with the transporter's lipid translocation (flip/flop and/or upward extrusion). The newly added TLC results appear as new Figure EV2 and are reproduced below (response 1.2)
It is important to note that there is significant ambiguity in the literature regarding the lipid translocation properties of ABCA extruders generally, especially for ABCA7. For example, it is unclear whether a mechanistic connection, if any, exists between lipid flipping and lipid extrusion by ABCA7. Takeda et al show a higher preference for PC/LPC transfer to apolipoproteins, while Quazi et al show greater ATPase activity for PS and PS flopping. Modulation of ATPAse activity only indirectly reports on substrate interactions for ABC transporters in general. Development of an assay that can quantitatively dissect these properties is a long-term goal of ours but is beyond the scope of the current manuscript. Both lipid flipping/flopping and extracellular extrusion require lipid entry in to the TMD. Our nanodisc incorporated structures of ABCA7 now offer a basis for what could not have been modeled based on published data and available structures previously. Our data allow a more detailed mechanistic model for lipid translocation (see response 1.4).

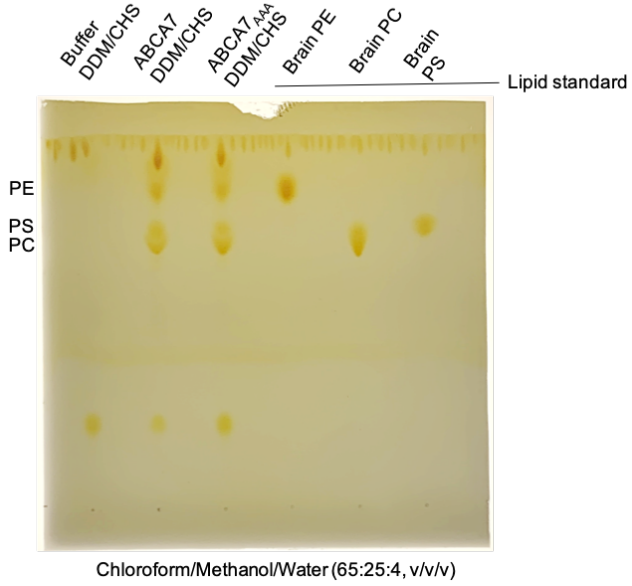
2) Figure 1E highlights a number of positively charged residues which appear to interact with the head-groups of the phospholipids. Some of them seem to be mutated in patients suffering from AD (line 209, R475 being mutated in AD patients). I see it as a missed opportunity that the authors did not attempt to mutate 2-3 of these positively charged residues into alanine and performed at least purifications, nanodiscs reconstitution and ATPase activity assays. What would be even nicer are some transport assays with such mutants. In any case, it would help the story if it contained some data going beyond structural descriptions (having said this, the structural descriptions are very well done with a right level of details!).

1.2 As per the reviewer's suggestion, we generated an ABCA7 variant harboring 3 mutations (R475A, K478A, and R482A) in the ECD, generated a stable cell line for its expression and purification (Figure EV2), and purified it for reconstitution into liposomes for comparing ATPase rates (Figure EV2 in manuscript). Liposomes were chosen for their unconstrained lipid environment compared to nanodiscs and for their higher ATPase rates. As shown below, this

triple A mutant ($ABCA7_{AAA}$) displays lower ATPase rates than liposomal wildtype ABCA7, further supporting a role for these positively charged residues in lipid interactions/translocation. We have also added TLC data analyzing the annular lipids co-purifying with both wildtype ABCA7 and $ABCA7_{AAA}$. We have added this information in the revised manuscript (line 127-134).



Comparison of SEC profile of $ABCA7_{AAA}$ and wildtype ABCA7 in DDM/C HS buffer (Left) and their ATPase activity in BPL/C h liposomes.



Annular lipid analysis for $ABCA7_{AAA}$ and wildtype ABCA7

Minor points

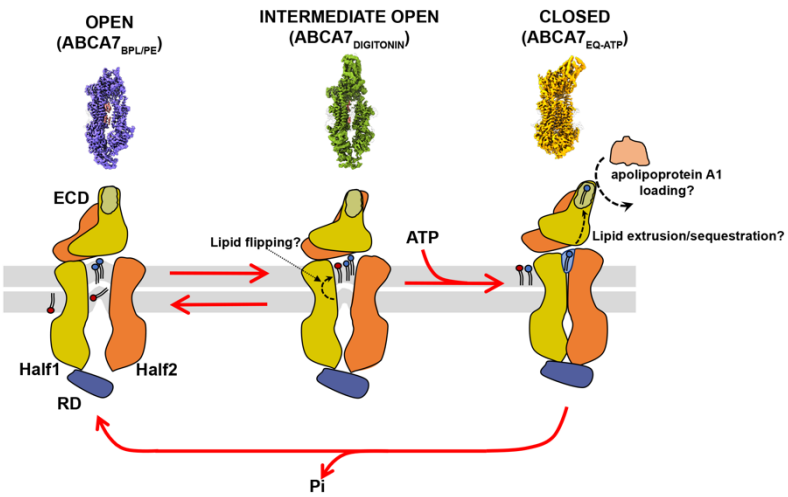
1) Figure 4 is hard to "read". In particular, one does not see the conformational differences very well in the very small overlay in Fig. 4A. The fact that the subdomains move as rigid body is

interesting (but not the main point in my view); rather, it is interesting to have captured ABCA7 in three main conformations. I am sure that with a bit of effort one can make this figure look nicer and more informative.

1.3 In our revision, we have updated the figure to show the overall conformational changes more clearly.

2) Likewise, I feel that Figure 6 (the mechanism figure) is too minimalistic. Such a thing might have been acceptable 20 years ago when we had no clue about ABC transport structure/function. The ABCA sub-family is by now pretty well-studied, with a good number of structures in the "game". Likewise, there are certainly some potential mechanisms that are currently discussed, among which of course the core questions on the whether these ABC transporters flip, flop or extract lipids! This should be somehow made visible. Next to fact that the ECDs likely play a major role.

1.4 We have updated our mechanistic model in Figure 6 to show 1) passage of ordered lipids in the fully open conformation, 2) the upward protrusion of the upper and lower leaflets that may aid in lipid flopping, and 3) the pocket showing lipids destined for extrusion to the ECD where they may be sequestered and reside until an acceptor molecule is available. The model highlights the ABCA7$_{DIGITONIN}$ structure as an intermediate state between the fully open and closed states. Combined with the bilayer doming effect seen in our MD simulations, this conformation may aid in the lateral transfer of lower leaflet lipid headgroups (flopping), with question marks to explicitly denote mechanisms where we can only speculate in the absence of direct experimental data. Our model can be contrasted with all other published ABCA transporter mechanistic models (ABCA1, ABCA3, and ABCA4) that invariably show two conformational states that are structurally very similar (with the exception of the ECDs) to rationalize lipid flipping (ABCA4) or flopping (ABCA1 and ABCA3) with no clear explanations for how opposite directions for lipid translocation occur.



Referee #2:

Le et al report the single particle cryo-EM structures of ABCA7 in the nucleotide-free and nucleotide bound state at resolutions of 3.6 and 4.0 Å, respectively. In the open, nucleotide-free state, lipids are present corresponding to the position of the inner and outer leaflet of the bilayer. Interestingly, no lipids were detected in the ATP-bound state that corresponds to the slightly outward-open state of ABCB7.

Structures of ABCA1 and ABCA4 have already been described. New in these structures is the presence of a lipid belt in the apo state, which is absent in the outward-open state and allows proposing of a model of lipid transport / flipping of ABCA7.

Overall the manuscript is in principle suitable for EMBO Journal, although a couple of points have to be addressed prior to a final decision.

We thank the reviewer for the positive evaluation of our study.

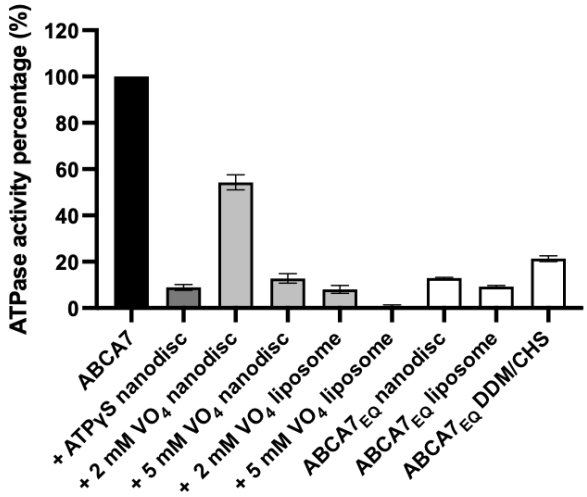
I listed my points / concerns in order of appearance and not importance:

- Line 63: The authors should provide a Table in SI summarizing KM and vmax values of ABCA7 in different environments. Also, what means drastically reduced ATPase activity for the EQ double mutant? A quick look at Figure S1A seems to tell me that the EQ has some 15% residual activity. This is substantial activity for a catalytic base residue! And higher as in the presence of ATPgammaS. This has to be clarified.

2.1 In light of the reviewer's comment, we have added a table of all K_M and V_{Max} values in Appendix Table S1 as well as a comparison of ATPase rates between wildtype ABCA7 and the EQ mutant for detergent, liposomes, and nanodiscs in Appendix Figure S1A. Residual ATPase activity of the EQ mutant is not unique to ABCA7 and has been reported for multiple human ABC transporters including ABCB1, ABCG1, ABCD1, and ABCD4. This fact suggests that the Gln sidechain can partially compensate for that of the missing Glu residue.

	Nanodiscs	Detergent (DDM/CHS)	Liposomes
K_M	0.51	0.43	0.74
V_{Max}	37.41	162.9	167.8

Table of Km and Vmax values (Appendix Table S1 in revision).



ATPase rates for ABCA7 under various experimental conditions as a percentage of wildtype ABCA7 ATPase activity in lipids, nanodiscs, and detergent.

- Figure S1A: I only see one bar for the EQ mutant, but the text reads for me that activity as determined in nanodiscs and detergent.

2.2 We apologize for the oversight and have added the requisite data for the EQ mutant in nanodiscs, liposomes, and detergent (see response 2.1)

- Line 75: I would suggest to recommend the statement concerning cholesterol. Data is presented only for nanodiscs (ND). Thus, I would claim an inhibitors effect only for ND or repeat he experiments in other environments.

2.3 We changed the sentence to read "cholesterol has an inhibitory effect on the ATPase activity of ABCA7 at least in nanodisc reconstituted samples."

- Line 95: A rmsd of 0.3 Å is identical and not 'nearly identical' at this resolution.

2.4 We agree and have changed this to "identical".

- Line 102 - 104: What is the driving force of lipids to entry the hydrophilic cavity of ABCA1?

2.5 We are unsure as to what hydrophilic cavity in ABCA1 the reviewer is referring to specifically. For ABCA7, the TMD cavity has a hydrophilic character at both the cytoplasmic and exoplasmic peripheries where lipid headgroups are most likely to reside. Interestingly, cavity residues of TMD1 and TMD2 reveal marked differences, with TMD1 being more electropositive, which may explain the observation of greater lipid association with TMD1 in our ABCA7 nanodisc structures. The central cavity retains a hydrophobic character consistent with occupancy by lipid acyl chains. As such, the driving force is likely a combination of favorable lipid interactions with the TMD as well as the lipid rich and fluid bilayer environment within the cavity, which allows lipid entry and exit upon TMD opening and closing, reminiscent of a

bellows-like mechanism where lipids would be 'sucked in' to the TMD in the open conformation and pushed out through exits both up towards the ECD and back out into the bilayer leaflet in the closed conformation.

- Line 117 - 118: As expected, ABCA7 in detergent does not show lipids in the cavity. What is the rational to claim that the lipids in ND are specifically bound to the protein and not simply a result of lipid crowding in the ND?

2.6 At this stage we cannot categorically dismiss any crowding effect induced by the nanodisc. Our protocol for nanodisc preparation entails using bulk lipids pre-incubated with the protein before the addition of MSP and prior to removal of detergent. Importantly, the transporter occupies a central position within the nanodiscs with no observed distortions of the nanodisc belt surrounding it. Furthermore, as can be clearly seen in Figure 1F (top and bottom panels), the observed lipids are continuous with the surrounding bilayer in a way that we speculate would not hinder their diffusion within the membrane (related to the reviewer's next point below). That said, we agree that the nanodisc environment likely provides a less fluid system compared to liposomes that could have helped resolve the ordered lipids in the TMD in our structures. It is important to note that nanodiscs are widely used as close membrane mimetic systems for structure-function studies. We are not aware of any ABC transporter structures in nanodiscs with similar lipid patches observed in the membrane despite the fact that many of the structures reveal large TMD lumens with lateral openings to surrounding bilayer (Nosol, Romane et al. 2020, Olsen, Alam et al. 2020, Le, Thompson et al. 2022).

- Line 120 - 121: Lipids in the ND are free to diffuse. In other words, they are less ordered and can be seen only at lower contour level. Again, this is no argument for specificity in my opinion. I would expect to generate mutants of for example the positively charged cluster, which interacts with the lipids. This would be a strong support of specificity, If a chance is observed at all.

2.7 The first point has been addressed in response 2.6 above and is consistent with our assertion that the open ABCA7 TMD provides access to lipids that, as the reviewer recognizes, are inherently more ordered than those outside the TMD. Whether specificity for individual lipid species exists within this framework is a separate topic and does not take away from the physiological relevance of the ordered TMD lipids seen in our structures. For the reviewer's second point, we generated a triple mutant of ABCA7 bearing R/K→A mutations in the ECD ($ABCA7_{AAA}$, see point 1.2 above for description), which shows reduced ATPase rates in liposomes, presumably due to a change in lipid interaction linked to ATPase activity modulation since these changes are far from its NBD catalytic sites. It is important to note that we only assert that the ABCA7 TMD lumen is accessible to a patch of ordered lipid bilayers, which is of significant physiological relevance and highly novel. We do not, at this point, suggest any quantification of substrate specificity. In our $ABCA7_{DIGITONIN}$ structure, the observed densities attributable to detergent are less ordered than the surrounding detergent micelle (opposite to our observation for the nanodisc structure containing lipids).

- Line 157: Is a translation of 2 Å at this resolution really something one should argue with?

2.8 A translation of only 2 Å, when combined with a 9° rotation for a rigid body like movement that consists of all residues from both the TMD2 and NBD2 domains, which occupy a large volume, leads to an overall substantial displacement that is substantial. These movements are well supported by our maps.

- Line 170: Please provide the rmsd values for the comparison.

2.9 A table of RMSD values for these rigid body comparisons discussed nearby line 197-206 between ABCA7 and ABCG2 & ABCG5/G8 were added to the bottom of Figure EV3B.

- Line 177: Can the MD simulations explain an inhibitory effect of cholesterol? It should bind to the protein.

2.10 At this stage, MD simulations cannot explain this effect. We speculate cholesterol could compete with lipid binding within the TMD, and we cannot, at this resolution, exclude partial occupancy of cholesterol within the density features that we modeled as acyl chains.

- Line 202: I wonder whether ABCA7 has a lipid specificity? According to the proposed model, ABCA7 would pump every lipid present in the inner leaflet to the outer leaflet. But this would abolish any lipid gradient such as the well-known PS gradient. Or am I missing something here? The same line of arguments holds for line 216- 217.

2.11 Please see our updated model (response 1.4). While we detected endogenous PE, PC, PS by TLC (see response 1.2) by co-purifying with ABCA7, we cannot make any claims regarding the binding of specific lipids. Therefore, in our structures we modeled only acyl chains into these density features. We agree with the reviewer that ABCA7 may alter the localized membrane asymmetry, which we speculate is part of its physiological role in endocytosis – it can be associated with enriched PS in the outer membrane in line with our proposed model. It is also worth noting that lipid flipping/flopping is distinct from lipid extrusion from the outer leaflet. The development and deployment of assays capable of dissecting these two mechanisms are beyond the scope of the current manuscript. Finally, likely ABCA7 acts in concert with the broader cellular lipid homeostasis machinery, and we speculate its effect may be strictly localized to its immediate membrane environment.

- Figure S9 and S10: I think that three sequence are truly not sufficient to make a claim that residues are conserved. More sequence can easily be incorporated and analyzed.

2.12 We modified our discussion to clarify that these residues are conserved within these transporters and added ABCA3 to our alignment file (Appendix Figure S7).

- Line 220: If ABCA7 flips PS (ref 16) again where is the specificity of flipping coming from? And this relation structural findings imposed on biology is my major concern with the manuscript.

2.13 The biological relevance of our findings lies in providing insights into lipid entry into the TMD and lipid translocation, both for lipid flipping (doming effect in the lower leaflet) and lipid extrusion (doming effect in the upper leaflet and extrusion pocket). Within this mechanistic framework, specificity may indeed exist, but in the absence of experimental support, we limit our discussion of this aspect, as stated above in response 2.11. It is important to note that ABCA4 is purported to carry out lipid flipping from the outer to inner leaflet even though the structures of ABCA4 in open and closed conformations look nearly identical to those of ABCA1, ABCA3, and ABCA7 (Figure EV5 in revision). By extension, we cannot exclude that lipid flipping (outer leaflet to inner leaflet) could also occur for ABCA7. Lipid depletion from the lower leaflet is also unlikely given the action of other lipid flippases known to be present in the cell membranes where ABCA7 is active, and its effect is likely restricted to its local membrane environment.

- Line 321: if 2 mM ATP was used for the purification of the EQ mutant, one could calculate whether a 15% residual activity of the EQ double mutant would hydrolyze sufficient amounts of ATP as the author state that ATP is bound to the NBD.

2.14 The density features are compatible with the presence of the gamma-phosphate group of ATP (Figure 3E). This observation can be contrasted with recent structural studies of ABCC8, where the consensus nucleotide binding site is suggested to have bound ADP compared to ATP at the degenerate site with density features that vary accordingly.

- Line 357: What concentration of orthovandate was used? How was it prepared. This is important in light of the efficiency of vanadate trapping.

2.15 We added additional data for vanadate trapping (See response 2.1). The following method to prepare vanadate was used - Sodium orthovanadate 100 mM stock: sodium orthovanadate powder was dissolved in water and its pH adjusted with hydrochloric acid to pH 10. The solution was boiled until it turned colorless. The process of pH adjusting was repeated several rounds till a stable pH in a colorless solution was obtained. We repeated our experiments with freshly prepared vanadate and have added these data in Appendix Figure S1A. In short, vanadate trapping is more efficient in liposomes (residual ATPase activity of 8.01% and 0.18% at 2mM and 5mM vanadate, respectively) compared to nanodiscs (residual ATPase activity of 54.29% and 12.78% at 2mM and 5mM vanadate, respectively), which could be related to the overall lower ATPase activity in nanodiscs.

- Figure S1A: Why is the trapping efficiency of vanadate so poor? Approximately only 1/3 of the activity is lost, but vanadate is known to be an efficient inhibitor of P-loop ATPases!

2.16 See response 2.15 above

Referee #3:

The manuscript by Le et al. describes a structural study on ABCA7 combining cryo-EM, ATPase assays and MD simulations. The study revealed the structure of ABCA7 in three conformations. The topic of the study is interesting and could contribute to our understanding of ABC transporter function, importantly to the structurally understudied ABCA family, but

unfortunately, the manuscript shows several shortcomings.

Claims are not supported by the data provided in the results section.
- The manuscript starts with a very promising title "Capturing the interplay of membrane lipids and structural transitions in human ABCA7", but it can't maintain the claim. The manuscript shows cryo-EM structure, which are static and it reports MD simulations, in which the protein is fixed and can't move, thus no transitions can be relieved. Author should find a title which better describes the content of manuscript.

3.1 We thank the reviewer for the title suggestion. We observe multiple states of the transporter associated with its transport cycle. While no real-time conformational dynamics are available from these structures, they do allow us to comment on the molecular rearrangements (transitions) that bring about these conformational changes and to map out the lipid-protein associations therein. The decision to restrain atomic coordinates during MD was necessary to prevent large deviations within the protein during the course of simulation that would not be supported by experimental data. Our goal here was simply to show that in the fully open conformation observed in our experimental ABCA7 nanodisc structures an entire patch of ordered bilayer lipids can occupy the TMD cavity. The MD data shed some light on possible amino acid sidechain interactions between lipid headgroups that were left unmodeled in our cryo-EM structures (see Figure SEV4D,E for contact plot). Taken together, these data suggest how the lipid bilayer membrane may be altered as a function of the structural transitions in ABCA7. In light od the reviewer's comment, we changed the manuscript's title to "Insight into the interplay of membrane lipids and conformational transitions in human ABCA7".

- It is not clear how the presented data would have a broader impact on the ABCA family, as the wide separation between TMD1 and TMD2 seems limited to ABCA7.

3.2 We have added a statement clarifying that the observed TMD conformation in nanodisc structures of ABCA7 may be unique to ABCA7, although we still maintain other ABCA family members in nanodiscs with similar MSP/lipid compositions may well show similar conformations based on the observed sequence and structural similarities (Appendix Figure S7 and Figure EV5). The broader impact derives from the fact that our structures provide a rationale for how outer leaflet lipids would enter the TMD and be extruded (in line with what has been recently proposed for ABCA1 as well (Segrest and Aller, 2022, ref 43 in revision), as well as how lower leaflet perturbations may aid in flipping lower leaflet lipids to the outer leaflet as showcased by our MD simulations.

- In the introduction (line 32-34) the authors make the claim to explain the substrate transport cycle. The results to not show new evidence to support the claim.

3.3 We respectfully disagree with reviewer here. This statement seems to completely ignore the fact that an ordered bilayer patch within the TMD has not been observed for any of the other published ABCA family structures. As such, our structures provide fundamental insight into how lipids (substrates) enter the TMD lumen and how individual lipids may remain in an extrusion pocket upon TMD closure for extraction. Moreover, the MD simulations show a possible trajectory for bilayer lipids as they enter the TMD that supports our experimental results.

- Line 40-43, the claim to understand a large number or diseases is far fetched.

3.4 In light of the reviewer's comment we have modified the relevant sentence to read:
Lines 40-43: 'To date, no direct structural information exists for ABCA7, and molecular insights into how its dysfunction alters inflammatory and immune responses, lipid homeostasis, and phagocytosis, which all contribute to AD progression are missing.'

- Line 53-55, the author incorrectly promise to directly visualize conformational changes associated with lipid partitioning. In reality, in the MD studies, the conformation of the protein is fixed by position restraints, thus these changes cannot be investigated with the presented simulations, while in the cryo-EM data, a direct and causality-linked structural response to lipid partitioning has not been shown. Also, line 92-96 the authors state, that the change in lipid composition had no structural effect, which seems to contradict the statement in line 53-55.

3.5 We apologize for the miscommunication. As described in our response to point 3.1 above, we observe multiple conformational states of the same protein that allow us to map out the associated molecular rearrangements (transitions). In the open state, lipids are observed in the TMD. In the absence of exogenously added lipids, an altered conformation ($ABCA7_{DIGITONIN}$) is observed for ABCA7. In the closed state, the TMD sterically occludes lipids ($ABCA7_{EQ\text{-}ATP}$). The MD simulations, which focus on describing lipid behavior around experimental structures, allow us to model the trajectory of lipids into an open TMD cavity. Constraining the protein coordinates during MD simulations was necessary to prevent large scale deviations that are not supported experimentally. Regarding the change in lipid composition, we observe the same cryo-EM structures in PE/Chol and BPL/Chol nanodiscs, although the former shows more ordered 3D classes and less conformational heterogeneity in 3D classification. We find no contradiction between the two points, as the section referred to as lines 53-55 pertains to the conformational changes associated with the presence or absence of lipids rather than differences within different lipid species.

- Line 223-224: I can't see the significant progress in our mechanical understanding ... . The structures are new and important, but the progress at the level of transporter mechanics it not become clear from the current manuscript.

3.6 We appreciate the reviewer's recognition of our structures being new and important and refer them to reviewer response 1.4 above that details the novel aspects of our results that have led to the formulation of a more detailed mechanistic model and have revamped our discussion accordingly.

-Information on number of repeats, concentrations, conditions, biochemical and statistical analysis is frequently missing for data shown in Fig1 and FigS1.

3.7 Information on number of repeats and error bars appears in each figure legend, whereas protein concentrations used in the experiments is detailed in the Methods section as well as in the source data file.

-ABCA7 has been proposed to be a PS transporter, much less of PE or PC. Please seen doi.org/10.1074/jbc.M113.508812 as an example. How can Fig1B be reconciled with this information? Could you give any reason, why the ATPase activity of PE is much higher then for PS.

3.8 The reviewer's point highlights one of many unresolved aspects of ABCA transporter physiology. ABCA7 was previously suggested to flop PS by Quazi et al (ref 16 in revision), which is distinguished from its preferred extracellular transport of PC and LPC shown by Tomioka et al (Ref 17 in revision). Our own published work shows significant alterations in brain lipid and fatty acid profiles in heterozygous knockout mice of ABCA7 (ref 13 in revision) especially in PE and PC, which is consistent with our data indicating a strong PE effect on ATPase activity. The smaller PE may have freer passage in and out of the TMD, allowing for more rapid TMD closure linked and, considering the rigid nature of the linked TMD-NBD conformational changes observed in our structures, a more rapid NBD dimerization could explain the higher ATPase rates seen for PE. We have added a discussion of this point in our discussion (see line 266-271).

-Vanadate trapping is probably the most widely used treatment to block ATPase activity across the ABC transporter superfamily. In Fig S1A, vanadate treatment reduces ATPase activity by less the 50%. Is this expected? Can this behaviour be explained?

3.9 We have now repeated our vanadate trapping experiments. In short, Vanadate trapping is more efficient in liposomes (residual activity of 8.01% and 0.18% at 2mM and 5mM Vanadate, respectively) compared to in nanodiscs (residual activity of 54.29% and 12.78% at 2mM and 5mM Vanadate, respectively), which could be related to the overall lower ATPase activity in nanodiscs. These data now appear in Appendix Figure S1 of our revision and are reproduced above in Response 2.1.

.
Simulation data:
-Which version of the Martini force field was used?
3.10 Martini 2.2 has been used in all the performed simulations. The Methods section has been updated to include this information in the revised manuscript.

Revised text: The resulting all-atom (AA) model was then converted to a coarse-grained (CG) Martini 2.2 model using the Martinize protocol (http://www.cgmartini.nl/), using an elastic network on atom pairs within a 10-Å cutoff

-For direct comparison between cryo-EM data and simulation data, these should use the same conditions. Currently, the cryo-EM - PE structure is cholesterol free, while the simulation employ a PE:cholesterol mixture.

3.11 We apologize for the confusion here. The Cryo-EM conditions with PE also contained 20% cholesterol. This has been further clarified in the text of our revision (line 374).

-I do not fully understand the dome-shape statement. Figure 5C,D and Fig S8A,B suggest this is actually not not the case. At the extracellular leaflet, the highest elevation is at the edge of the TMD-lipid interface and extends as much into the membrane as into the cleft between TMD1-TMD2. Second, this is only visible at one side of the transporter. At the cytoplasmic side, the 2D plot suggest the opposite of a dome, as the centre is further away from the centre of the membrane. This is seen for both PC and PE. Given that the PE lipids create strong membrane curvature, while a PC/Chol membrane is in its biophysical properties closer to and eukaryotic cell membrane, I would suggest to flip the PE and PC dataset between Fig 5 and Fig S8.

3.12 We thank the reviewer for the comment. Firstly, as per reviewer's suggestion we have flipped the PE and PC datasets between Figure 5B,C and EV4A,B and the corresponding figure panels have been updated. As far as the dome shape is concerned, this region is generated by lipid elevation (moving toward +z) which also results in the thickening of the outer leaflet and thinning of the inner leaflet in the lumen of TMD (by 5-10 Å in each leaflet). In Figs 5B,C and EV4A,B we do see the formation of a lipid-elevated region (yellow color in the heatmaps) in the vicinity of TMD and in both leaflets, therefore, the formation of a dome-shaped lipid structure. However, to follow the reviewer's suggestion we have changed the dome-shaped terminology to lipid elevation in the revised manuscript. Moreover, further explanation has been added to the results to avoid any confusion in the interpretation of data.

Updated text in the manuscript: We observed a distinct elevation of lipid headgroups in both bilayer leaflets within the TMD, causing the thickening and thinning of the outer and inner leaflets, respectively.

-The results from the quantification of physpholipids is hampered by the fact that the protein Ca atoms remain fixed in the conformation observed in the cryo-EM structure. Therefore, ABCA7 cannot move or dynamically react on interactions of lipids, while lipids might be force to adopt distributions, which are only induced because the geometry of the restrained transporter. At least, after the 2 μs, the simulations need to be extended for sufficient time without restraints.

3.13 While we agree with the reviewer regarding the side effect of our simulation setup, restraining the C_α atoms during MD simulations was intentional to preserve the experimental cryo-EM structure of the protein and study membrane reaction to those structures, which, despite its imperfections, is a common practice in studying lipid-protein interaction studies (see, e.g., https://doi.org/10.1021/acscentsci.8b00143). Our multi-microsecond MD simulations were able to capture the lipid penetration in the TMD of the captured cryo-EM conformation of ABCA7. We have added more clarification to the Results and Methods sections of the manuscript.

Revised text:
Results: we performed multi-μs MD simulations of the open conformation of $ABCA7_{PE}$, with positional restraints on protein C_α atoms to maintain the conformation captured in our cryo-EM structure.

Methods: Each system was then simulated for 2 μs, with restraints only applied to the protein backbones (to maintain the cryo-EM captured conformation), resulting in an aggregate sampling of 8 μs (4 copies × 2 μs).

-The preference of partitioning of PE over PC is uncertain. This is difficult to reconcile with the data shown in Fig 5. Fig 5A/insert, which would not indicate the preference. Also, Fig 5B does not support the statement, because the standard deviations are strongly overlapping. It would be helpful to show the actual distributions histograms or the time evolution individually for each of the four transporters, as the bar graph is hiding some of important details.

3.14 We thank the reviewer for the suggestion. We now have added a new figure to SI (Appendix Fig. S6), presenting the raw data on the lipid counts in the lumen of the protein for both POPE and POPC systems, separately for each protein copy. As the reviewer indicated, we observe similar patterns for POPE and POPC systems for three out of four copies. Only copy IV of the POPE system shows a higher range of lipid penetration into the lumen of ABCA7. We have therefore removed the statement about POPE preferential inclusion from the revised manuscript, and only present the raw data in the figures.

Other

-The idea of an exit pocket is interesting, but has not been explored beyond suggesting its existence and reporting its geometry. The link to the loop containing residues 475, 478, 482 as well as 678 could be better explored, but current simulations are limited by the position restraints.

3.15 We have added ATPase data for an ECD mutant ($ABCA7_{AAA}$) that further highlight the role these residues may play in lipid translocation based on reduced ATPase rates of the mutant.

-Figure 4E suggests a larger intradomain motion of the NBDs of the α-helical domain vs the recA-like domain, which was observed before in several other ABC transports. If true, this could be explored, but would also imply that the statement, that all motions are rigid body motions, is not correct.

3.16 We now clarify the NBD movements associated with the different structures and focus our discussion of rigid body movements largely on the TMDs, although the NBD-TMD pairs do appear to move as rigid bodies in the fully open $ABCA7_{BPL/PE}$ and intermediate open $ABCA7_{DIGITONIN}$ structures, which may be important in coupling conformational transitions between the two domains till the point of NBD dimerization (Lines 188-190).

-Line 175-176: The fact that ABC transporter show a large contribution of rigid body motions as shown here is not novel, but well established.

3.17 While we agree rigid-body motions have been observed for other ABC transporters, those outlined here for ABCA7 and their comparison to ABCG family members to showcase their general conservation (for the TMDs specifically) among Type V ABC transporters is indeed

novel. These rigid-body movements are also important to emphasize in light of our data showing bilayer lipids within the TMD whose extrusion would be facilitated by the rigid nature of the TMDs.

-The comparison to the ABCG is interesting, but more important is a comparison to the closely related ABCA1 and ABCA4 structures and conformations, which both have been solved in more than one conformation.

3.18 The comparison with ABCG family members establishes a broader conservation of the rigid-body movements in type V ABC transporters, whereas TMD-NBD conformations within the available ABCA transporter structures in detergents are nearly identical. Only our nanodisc structures revealing the larger, lipid-filled TMDs are unique. The transitions in between based on an extrapolation based on end states visualized in the structures suggest rigid-body motion. ABCA7 shares a very similar overall architecture in its intermediate open conformation in detergent ($ABCA7_{DIGITONIN}$) as that of ABCA1, ABCA3, and ABCA4 (all determined in detergent) as well as in its closed conformation ($ABCA7_{EQ\text{-}ATP}$) as those of ATP bound, closed structures of ABCA1, ABCA3, and ABCA4 (Sun et al, 2022; Xie et al, 2022; Xie et al, 2021; Liu et al, 2021). We have clarified this further in the text (line 283-288) and added a new figure with the relevant structural alignments (Figure EV5).

-I do not understand the use of the ABCB family transporter TM287 during the model building process. It has a has a different TMD fold. Please explain. Also, citation 51 is not citing any work on TM287.

3.19 We apologize for the confusion. Only the NBDs from TM287 (which are structurally conserved among the different ABC transporter families) were initially used to guide NBD modeling in ABCA7. This has been clarified further as only the C alpha trace was used as a rough guide to compare our observed density features of the open-conformation ABCA7 structures. Our models were further compared to alphafold predictions, ABCA4 structures that came out subsequently, as well as our own $ABCA7_{EQ\text{-}ATP}$ given its better resolved NBD helical and recA like domains.

-The model of Fig 6 is incomplete for my understanding: The RD region holds the ABCA7 dimer together at the cytosolic site, the ECD at the extracellular side. Also, the model shows a lipid half flipped inside the transporters. Is there any evidence for this speculation from MD or cryo-EM? It is also not described in text, if the authors assume that the substrate lipid would change orientation within the transporter. It could e.g. as well occur in the exit pocket.

3.20 The model has been updated to include the ECD and RD (See point 1.4 above). The half-flipped lipid is based on MD results that show the acyl chains orientations of lower leaflet lipids.

-For reporting on lipid transport and interaction with the transporter, simulations will need to include PS lipids, which according to current knowledge, are the most likely substrates.

3.21 Our MD simulations were conducted to show lipid partitioning into the TMDs in the new, open conformation observed in our ABCA7 structures. PE and PC were chosen because they represent the two extremes of modulatory effect for our ATPase data. As such, we do not feel that addition of additional PS simulation data would add further to our mechanistic conclusions.

Minor points:
line 62: It should read "Michaelis-Menten constant" instead of "Michaelis constant".

3.22 "Michaelis-Menten" refers to the kinetics of the reaction, whereas K_M is generally referred to as the "Michaelis constant". As such, we do not think the suggested change is warranted.

line 63: Please provide exact numbers for the Km in the different conditions.

3.23 This information has been added as supplementary Table EV1 and reproduced in response 2.1

line 89: Should it read TMD1-ECD1 or TMD2-ECD1. Please check.

3.24 The sentence is correct as is meant to convey that TMD1 shares a greater interface with ECD2 compared to ECD1.

line 99: Is ABCA7BPE correct? Please check.

3.25 We apologize for the confusion. BPE refers to Brain Phosphatidylethanolamine. However, for clarity, we now only use $ABCA7_{PE}$ to refer to the sample of ABCA7 reconstituted in Brain Phosphatidylethanolamine : Cholesterol nanodiscs.

line 209-211. A deformation of the membrane plane will require energy input. If it is not caused by the position restraints in the first place, this deformation will very likely serve as an energetic and kinetic barrier instead of aiding (accelerating) binding of lipids, as proposed.

3.26 The deformation can be easily induced by the proteins embedded in the membrane, as reported in numerous examples in the literature. The polarity and charge of different residues that are exposed to lipid bilayer (as shown in Fig. 5D) cause the lipid structural changes in the vicinity of proteins, in this case ABCA7. We believe this lipid deformation (mainly lipid elevation in the lumen) might assist lipid transport by facilitating their initial translocation toward the extracellular gate.

Methods: please confirm that HEK293 cells were used for WT ABCA7, while HEK293T cells were used for the mutants.

3.27 HEK293T cells were used for all constructs for transient expression, whereas all stable cell lines were generated using the HEK293TREX system.

Figure 5: The colour legend on panel C/D are very difficult to identify. Please use a clearer representation

3.27 The figure has been updated.

Figure 6S: Please add residues labels

3.28 Residue labels have been added, as suggested.

Figure S8E: it is unclear, which type of protein representation is used.

3.29 The protein representation has been mentioned in the figure caption.

Dear Amer,

We have now received re-review reports from two referees. As you will see, you have addressed their concerns satisfactorily. Before I can finally accept the manuscript though, there are some remaining editorial points which need to be addressed. In this regard would you please:

- Make sure that the final text file (.doc) contains no figures and no track changes
- Include funding information for grant number MCA06N060 on our website
- Add up to five keywords
- Ensure that the references are in alphabetical order; in the text, please use 1 author + et al. For references with long author lists, please limit to 10 authors + et al. in the reference section

Rename the conflict of interest statement as the "DISCLOSURE AND COMPETING INTERESTS STATEMENT"

- Remove the AC/CrediT section from the text
- Complete the general info table in the checklist
- Add page numbers and Appendix figure S8 to the table of contents
- Reorganize Appendix figure Source data file to one file/folder per figure and zip

We include a synopsis of the paper (see http://emboj.embopress.org/). Please provide me with a general summary statement and 3-5 bullet points that capture the key findings of the paper.

EMBO Press is an editorially independent publishing platform for the development of EMBO scientific publications.

Best wishes,

William

William Teale, PhD
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We realize that it is difficult to revise to a specific deadline. In the interest of protecting the conceptual advance provided by the

work, we recommend a revision within 3 months (31st Jan 2023). Please discuss the revision progress ahead of this time with the editor if you require more time to complete the revisions. Use the link below to submit your revision:

https://emboj.msubmit.net/cgi-bin/main.plex

Referee #1:

The authors have addressed all my open questions and have made additional functional and biochemical experiments to further strengthen their work. I therefore suggest publication of this manuscript in EMBO Journal.

Referee #2:

All the points that I raised have been addressed adequately in the revised version of the manuscript. Having said this, I would support acceptance of the revised version.

2nd Revision - Editorial Decision **10th Nov 2022**

Dear Amer,

I am pleased to inform you that your manuscript has been accepted for publication in the EMBO Journal.

Congratulations on the publication of a very elegant study!

Please note that it is EMBO Journal policy for the transcript of the editorial process (containing referee reports and your response letter) to be published as an online supplement to each paper. If you do NOT want this, you will need to inform the Editorial Office via email immediately. More information is available here:
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Best wishes,

William

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This checklist is adapted from Materials Design Analysis Reporting (MDAR) Checklist for Authors. MDAR establishes a minimum set of requirements in transparent reporting in the life sciences (see Statement of Task: 10.31222/osf.io/9sm4x). Please follow the journal's guidelines in preparing your manuscript.
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Abridged guidelines for figures
1. Data
The data shown in figures should satisfy the following conditions:
- the data were obtained and processed according to the field's best practice and are presented to reflect the results of the experiments in an accurate and unbiased manner.
- ideally, figure panels should include only measurements that are directly comparable to each other and obtained with the same assay.
- plots include clearly labeled error bars for independent experiments and sample sizes. Unless justified, error bars should not be shown for technical replicates.
- if n<5, the individual data points from each experiment should be plotted. Any statistical test employed should be justified.
- Source Data should be included to report the data underlying figures according to the guidelines set out in the authorship guidelines on Data Presentation.

2. Captions
Each figure caption should contain the following information, for each panel where they are relevant:
- a specification of the experimental system investigated (eg cell line, species name).
- the assay(s) and method(s) used to carry out the reported observations and measurements.
- an explicit mention of the biological and chemical entity(ies) that are being measured.
- an explicit mention of the biological and chemical entity(ies) that are altered/varied/perturbed in a controlled manner.
- the exact sample size (n) for each experimental group/condition, given as a number, not a range;
- a description of the sample collection allowing the reader to understand whether the samples represent technical or biological replicates (including how many animals, litters, cultures, etc.).
- a statement of how many times the experiment shown was independently replicated in the laboratory.
- definitions of statistical methods and measures:
  - common tests, such as t-test (please specify whether paired vs. unpaired), simple χ2 tests, Wilcoxon and Mann-Whitney tests, can be unambiguously identified by name only, but more complex techniques should be described in the methods section;
  - are tests one-sided or two-sided?
  - are there adjustments for multiple comparisons?
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Please complete ALL of the questions below.
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Materials

Newly Created Materials	Information included in the manuscript?	In which section is the information available? (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section)
New materials and reagents need to be available; do any restrictions apply?	Yes	Materials and Methods: Protein purification, Thin-layer chromathography. Figures: Figure EV2.

Antibodies	Information included in the manuscript?	In which section is the information available? (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section)
For **antibodies** provide the following information: - Commercial antibodies: RRID (if possible) or supplier name, catalogue number and or/clone number - Non-commercial: RRID or citation	Not Applicable	

DNA and RNA sequences	Information included in the manuscript?	In which section is the information available? (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section)
Short novel DNA or RNA including primers, probes: provide the sequences.	Yes	A primer pair for generation E965Q using site directed mutagenesis by PCR. Materials and Methods: Protein purification.

Cell materials	Information included in the manuscript?	In which section is the information available? (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section)
Cell lines: Provide species information, strain. Provide accession number in repository **OR** supplier name, catalog number, clone number, and/**OR** RRID.	Yes	HEK293T cells for transient expression, HEK293TREX system for stable cell line generation. Materials and Methods: Protein purification.
Primary cultures: Provide species, strain, sex of origin, genetic modification status.	Not Applicable	
Report if the cell lines were recently **authenticated** (e.g., by STR profiling) and tested for mycoplasma contamination.	Not Applicable	

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If your work benefited from core facilities, was their service mentioned in the acknowledgments section?	Yes	Acknowledgments: cryo-EM and shared instruments core facilities at the Hormel Institute.

Design

Study protocol	Information included in the manuscript?	In which section is the information available? (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section)

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Report the **clinical trial registration number** (at ClinicalTrials.gov or equivalent), where applicable.	Not Applicable	

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Include a statement about **sample size** estimate even if no statistical methods were used.	Yes	Methods: ATPase assay, Figures: Figure 1, Figure EV2, Appendix Figure S1, Appendix Figure S3.
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For every figure, are **statistical tests** justified as appropriate? Do the data meet the assumptions of the tests (e.g., normal distribution)? Describe any methods used to assess it. Is there an estimate of variation within each group of data? Is the variance similar between the groups that are being statistically compared?	Yes	Figure EV2: (B) Statistical significance by unpaired, two-tailed t-test p-value of <0.0001 is indicated by *****

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Have **primary datasets** been deposited according to the journal's guidelines (see 'Data Deposition' section) and the respective accession numbers provided in the Data Availability Section?	Yes	Data and Materials avaliability: The cryo-EM Maps have been deposited at the Electron Microscopy Databank (EMDB) under accession codes EMD-28041 (ABCA7BPL Map 1), EMD-28044 (ABCA7BPL Map 2), EMD-28047 (ABCA7PE), EMD-28451 (ABCA7EQ-ATP), and EMD-28050 (ABCA7DIGITONIN). The associated atomic coordinates have been deposited at the Protein Data bank (PDB) under accession codes 8EDW (ABCA7BPL), 8EE6 (ABCA7PE), 8EOP (ABCA7EQ-ATP), and 8EEB (ABCA7DIGITONIN).
Were **human clinical and genomic datasets** deposited in a public access-controlled repository in accordance to ethical obligations to the patients and to the applicable consent agreement?	Not Applicable	
Are **computational models** that are central and integral to a study available without restrictions in a machine-readable form? Were the relevant accession numbers or links provided?	Not Applicable	
If publicly available data were reused, provide the respective **data citations in the reference list**.	Yes	References: Figure EV3, Figure EV5