ApoE4 upregulates the activity of mitochondria-associated ER membranes

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(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. The original formatting of letters and referee reports may not be reflected in this compilation.)

1st Editorial Decision 14 June 2015

Thank you very much for the submission of your research manuscript to our editorial office and for your patience while we were waiting to hear back from the referees. I apologize for the delay in getting back to you, but we have just now received the full set of reviews on your manuscript.

As the detailed reports are pasted below I will only repeat the main points here. Both reviewers 1 and 2 agree on the potential interest of the findings and, in principle, support publication of it in EMBO reports once their concerns have been addressed (either in writing or through additional experimentation as indicated in their reports). Referee 3 is more critical, as s/he feels that the use of fibroblasts is not an adequate cell type to study Alzheimer's disease. However, upon further consultation with the other referees and an additional advisor we agreed that we would not insist that you repeat some/all experiments in neuronal cells. However, if you already have such data, we, of course, encourage you to add them, but would not make this a prerequisite for publication.

Given the potential interest of your findings, I would like to give you the opportunity to revise your manuscript, with the understanding that the main concerns of referees 1 and 2 should be addressed. In addition, I would also encourage you to discuss in more depth the possible consequences of increased MAM function upon ApoE4 treatment for the pathophysiology of AD, as this remains unexplored at the moment.

Acceptance of the manuscript will depend on a positive outcome of a second round of review and I should also remind you that it is EMBO reports policy to allow a single round of revision only and that therefore, acceptance or rejection of the manuscript will depend on the completeness of your responses included in the next, final version of the manuscript. Please also note that our scientific
reports (as opposed to full articles) contain a combined results and discussion section and I would kindly ask you to modify the text accordingly before submitting the revised version. In addition,

Revised manuscripts should be submitted within three months of a request for revision; they will otherwise be treated as new submissions.

When submitting your revised manuscript, please include:
• a word-formatted version of the manuscript text
• editable, high-resolution TIFF or EPS-formatted or figure files
• a letter detailing your responses to the referee comments (as word file if possible)
• a short, two-sentence summary of the manuscript
• two to three bullet points highlighting the key findings of your study
• a schematic figure (in jpeg or tiff format with the exact width of 550 pixels and a height of about 400 pixels) that can be used as part of a visual synopsis on our website
• a complete author checklist, which you can download from our author guidelines (http://embor.embopress.org/authorguide#revision)

Expanded view figures: We are in the process of updating the way in which we display additional/supplementary information. In essence, all supplementary figures are now called Expanded View Figures and should be labeled and referenced as Figure EV1, Figure EV2 etc. in the main text of the manuscript. The legends for the EV figures should be incorporated in the main body of the text after the legends for the main figures. Please modify your additional figures accordingly.

As part of EMBO's Transparent Editorial Process, EMBO reports publishes online a Review Process File to accompany accepted manuscripts. This File will be published in conjunction with your paper and will include the referee reports, your point-by-point response and all pertinent correspondence relating to the manuscript.

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We have also started encouraging authors to submit the raw data of biochemical and/or microscopical images to our editorial office. These data will be published online as part of the supplementary information. This is voluntary at the moment, but I encourage you to supply these files when submitting the revised version of your study.

I look forward to seeing a revised form of your manuscript when it is ready. Should you in the meantime have any questions, please do not hesitate to contact me.

REFEREE REPORTS

Referee #1:

This is a very interesting manuscript reporting that the risk factor for AD ApoE4 increases MAM function respective to ApoE3. The authors ascribe the function to a role of Apo as part of the lipoprotein, pointing out to a specific role in metabolism. While the manuscript is not data-heavy, the experiments are technically sound and well controlled, and the topic is of major interest as it offers a framework to understand the role of ApoE4 as a risk factor for AD (needless to say, the molecular explanation for this association is still lacking).

I believe that the paper would be considerably stronger when the Authors address the following points

1. what happens to the effect of ApoE4 on CE synthesis in cells lacking Mfn2? according to the authors' prediction, it shall have no/reduced effect
2. I recommend that the authors also show the non treated samples for comparison. As the paper is presented, it is unclear if ApoE3 has per se some effect on lipid metabolism
3. How do ER-mito connections look like upon ApoE3/4 treatment? This is an important piece of evidence that is missing and shall be included.

4. What happens to enzymes of the three measured pathways in ApoE3/4 treated cells? Their levels shall me measured and shown.

Referee #2:

This well written paper describes an important and logical extension of a series of papers from the laboratory of Professor Eric Shon that have addressed an entirely novel theory for the pathogenesis of Alzheimer's disease, a disorder that is predicted to reach "epidemic" proportions, given demographic predictions of the growing proportions of aged individuals within the developed societies. That theory (the "MAM Hypothesis") invokes a primary locus of pathophysiology at the mitochondria-associated domain of the endoplasmic reticular membranes (MAM), resulting in downstream phenomena that define the disorder, notably the classical neuropathological diagnostic criteria of neuritic beta amyloid plaques and neurofibrillary tangles.

Shon and his colleagues first provided surprising evidence of the localization of Presenilin 1 and 2 and of their secretase functions within these MAM structures. They then demonstrated major increases in the MAM-associated synthesis of cholesteryl esters and phospholipids within cells bearing mutations in Presenilin (dominant mutations that lead to early onset, familial forms of Alzheimer's disease) as well as in cells from patients with the vastly more common late onset, sporadic forms of the disease. The present paper asks whether the major genetic risk factor for these common sporadic forms of the disease, the APOE4 allele of Apolipoprotein E, has similar impacts upon MAM functions. The data in the present paper clearly support this hypothesis.

I have only two major suggestions for the authors to consider, both of which could enrich the Discussion section of the manuscript. They would address two of the most interesting and important questions in this field, but questions that have not been given sufficient attention.

First of all, given that some yet to be defined process or processes associated with the biology of aging clearly pose the major risk factor for Alzheimer's disease, what is the state of the evidence that certain alterations of MAM function or functions evolve during the course of normative aging in the presence of various APOE alleles?

Second, given the evidence that the "bad" APOE4 allele is closest to the typical allele of non-human primates (Chimpanzees, for example, share with human apoE4 the two arginine (R) residues 112 and 158, PLoS One. 2012;7(10):e47760. doi: 10.1371); that two fossil DNA sequences of Denisovans appeared to be closest to APOE4 (J Mol Evol. 2014 Jun;78(6):321-3. doi: 10.1007/s00239-014-9628-x); that the population frequencies among some human populations exhibit comparatively high frequencies of that allele - what selective advantages of these altered MAM functions could these alleles have provided? Could that allele have provided selective resistance to infectious agents, for example, by limiting access to certain classes of lipids (J Biol Chem. 1995 Aug 25; 270(34):19791-9; Neurobiol Aging. 1999 Jul-Aug;20(4):441-3)? Do the authors have other suggestions on potential selective advantages of the several classes of MAM functions that are modified by the APOE4 allele?

Referee #3:

My apologies for my late review of this complex paper. I had other responsibilities which I could not delay, but I am sorry for holding up the process.

I do not agree with the introduction. AD is associated with an array of biochemical alterations, including perturbations in cholesterol homeostasis [2,3], phospholipid metabolism [4], calcium trafficking [5], and mitochondrial function [6].

While nearly every possible biochemical process has, at some time, been proposed by somebody to be involved in the disease process, this is nearly meaningless and an arbitrary selection of the literature. Genetic analysis has shown 3 processes: cholesterol metabolism, endosomal vesicle...
recrecycling and innate immunity. Mitochondrial dysfunction has not come out of any analyses for AD
(in contrast to PD where it is well established). This is an important point because it subverts the
rationale for the study. On the issue of cholesterol, the identification of ABCA7 mutations clearly
indicates cholesterol is likely to be the central issue.

However, my major concern with the work is the inadequacy of the model system. I do not think
that work in fibroblasts can convincingly give us insight into isoform specific neuronal biology and I
think this really undercuts the value of this work.

1st Revision - authors' response 28 September 2015

We thank the reviewers for their comments. All substantive changes are highlighted in yellow.

Comments of Reviewer #1:
1. "what happens to the effect of ApoE4 on CE synthesis in cells lacking Mfn2? according to the
authors' prediction, it shall have no/reduced effect"

The reviewer's prediction was correct. The differential effect of ApoE4 vs ApoE3 ACM was
abrogated upon treatment of Mfn2-KO MEFs. This result is now shown in Revised Figure 3B.

2. "I recommend that the authors also show the non treated samples for comparison. As the paper is
presented, it is unclear if ApoE3 has per se some effect on lipid metabolism"

While we understand the desire for a “non-treated” control, performing such an experiment cleanly
is actually conceptually quite difficult, as we would actually be introducing a second variable,
thereby making the results difficult to interpret. Specifically, if we add media without ApoE-ACM,
we are ignoring the effects that the ACM itself, independent of ApoE, have on MAM activity. We
tried to navigate around this issue by using ACM from ApoE-KO mouse astrocytes. However, when
we applied this ApoE-KO ACM to cells, it had an unexpectedly toxic effect (implying that
minimally, some amount of lipoprotein in the medium is essential for viability). The other
alternative - using ACM from mouse astrocytes - is equally problematic, because it contains mouse
ApoE, which differs from human ApoE, especially at a critical residue at position 61 (it is Thr in
mice but is Arg in humans). Thus, any effect seen (or not seen) using mouse ApoE ACM would be
hard to put into context. In any event, our main focus has been to elucidate the difference between
human ApoE3 and ApoE4, and we feel that we have accomplished that with the experiments
performed.

3. "How do ER-mito connections look like upon ApoE3/4 treatment? this is an important piece of
evidence that is missing and shall be included"

We agree with the reviewer that this is an important point, and have have included the data in new
Figure 4. We indeed found that the median number of cells with a high level of ER-mitochondrial
co-localization was greater in ApoE4-ACM treated cells than in ApoE3-treated cells, by confocal
microscopy, but the overall results did not achieve statistical significance. As discussed in the
manuscript, we note that all the functional effects of ApoE4 on MAM function reported here, while
indeed statistically significant, were, on an absolute level, lower those found in PS-mutant and AD
cells. This would be consistent with ApoE4's status as a risk factor, not a determinative factor, in
AD. Moreover, it may be that the methods that we used were able to "catch" the biochemical
alterations more easily than the morphological changes.

4. "what happens to enzymes of the three measured pathways in ApoE3/4 treated cells? their levels
shall me measured and shown."

We checked the levels of ACAT1 and PISD, two key enzymes involved in the biosynthesis of
cholesterol esters and phosphatidylethanolamine, respectively (we were not clear on the third
pathway the reviewer was alluding to). We found no difference between the levels of these enzymes
when comparing ApoE3 and ApoE4 ACM treatment of WT fibroblasts. The results are shown in
new Figure S2.
Comments of Reviewer #2

1. "First of all, given that some yet to be defined process or processes associated with the biology of aging clearly pose the major risk factor for Alzheimer's disease, what is the state of the evidence that certain alterations of MAM function or functions evolve during the course of normative aging in the presence of various APOE alleles?"

2. "Second, given the evidence that the "bad" APOE4 allele is closest to the typical allele of nonhuman primates, ... that two fossil DNA sequences of Denisovans appeared to be closest to APOE4, ... and that the population frequencies among some human populations exhibit comparatively high frequencies of that allele - what selective advantages of these altered MAM functions could these alleles have provided? Could that allele have provided selective resistance to infectious agents, for example, by limiting access to certain classes of lipids? ... Do the authors have other suggestions on potential selective advantages of the several classes of MAM functions that are modified by the APOE4 allele?"

The reviewer raises excellent questions that, although difficult to answer experimentally, have been addressed in the Discussion. It is interesting to speculate why the deleterious APOE4 allele would persist at such high frequencies in humans. Certainly, selective advantage in immunity would offer an explanation. This idea is bolstered by the experimental work of others, who have demonstrated that ApoE can modulate susceptibility to certain pathogens. How does MAM fit into this? We speculate that its role as a lipid raft and its control of the synthesis of the lipidic components of lipid rafts may influence infectivity of these pathogens, as the importance of lipid rafts for entry into the cell has been well established. As for aging and MAM function, there is a paucity of literature on the subject, though tautologically, one might expect MAM function to increase with age, as we see alterations in MAM function in age-related disorders such as AD and PD. In fact, we are looking at MAM function in normal fibroblasts of different ages (both age of the subject and passage number), but that work is only in its preliminary stages; we hope that it becomes the subject of a future publication.

2nd Editorial Decision
21 October 2015

I am very pleased to accept your manuscript for publication in the next available issue of EMBO reports. Thank you for your contribution to our journal.