Author's response to reviews

Title: Atherosclerosis and Alzheimer - diseases with a common cause?
Inflammation, oxysterols, vasculature

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Author's response to reviews: see over
Author Revisions and Response to Reviewers

Atherosclerosis and Alzheimer - diseases with a common cause? Inflammation, oxysterols, vasculature
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{Responses and revisions are given below in blue font}

We welcome the positive and constructive comments of both reviewers. In the following we (i) respond to each comment, (ii) detail the changes we have made in response to the suggestion of the reviewer, and (iii) summarize other minor revisions we have made in response to comments from other colleagues.

Version: 1 Date: 11 November 2013
Reviewer: David W Russell
Level of interest: An article of outstanding merit and interest in its field

Reviewer's report:
The hypothesis that Alzheimer’s Disease and atherosclerosis share a common etiology associated with infection is developed. An extensive, comprehensive, and fair review of the literature is used to compare and contrast similarities and differences between the two diseases and to arrive at the conclusion that both appear to be exacerbated by infection.

We thank the reviewer for this very positive assessment as well as for drawing our attention to some new and highly relevant findings.

Specific Comments
1. On page 9, reference is made to the LDL receptor-related receptor; is this the same protein as LRP1, which is referred to later in the manuscript?

We stand corrected: we had used out-of-date nomenclature; these are the same molecule (full name low-density lipoprotein receptor-related protein 1; official symbol LRP1). We have now added the full name and symbol at first mention, and refer subsequently to the official symbol for the protein.

2. Is there any evidence that heterozygous carriers of LDL receptor mutations (familial hypercholesterolemia, FH) have an increased incidence of dementia, or do these individuals not live long enough to become susceptible to neurodegeneration?

Hypercholesterolemia is clearly a risk factor for AD development. Few studies have addressed the specific issue of LDLR mutations, and for precisely the reasons the reviewer suspected – Zambon et al. (Am. J. Med. 123, 267–274, 2010) noted "subjects with the highest levels of cholesterol generally die at younger ages from cardiovascular events and are lost from the samples of elderly subjects (i.e., comparative studies of Alzheimer’s disease patients versus controls), introducing a bias known as survivor effect". They looked instead at early signs of dementia in a
cohort of patients with LDLR mutations, and reported a seven-fold increased incidence of mild cognitive impairment in LDLR mutations.

We have added a note to the text to emphasize this point.

3. Page 13, line 14: APOE knockout animals

We thank the reviewer for noting this typo; this has been corrected.

4. Curcumin is often used as an inhibitor of NF-kappaB and relative to this article, has been shown to block induction of cholesterol 25-hydroxylase (ref. 283).

This is a very insightful comment. Although one worries about the specificity of NF-κB inhibition by curcumin, there is no doubt that Bauman et al. (former ref. 283) do indeed demonstrate that curcumin can abolish CH25H induction. We have added discussion of this very important result to the text.

5. T-Y. Chang and colleagues have published papers (PNAS 107:3081 and Mol Ther 21:1497) indicating that knockout of ACAT1 is associated with a marked reduction in amyloid deposition; these should perhaps be added to the section on page 29 in which ACAT inhibitors are discussed.

We were not aware of the latter Mol Ther report (published after submission); the former paper was only cited in Table 1. The Mol Ther paper is indeed important because it confirms a specific role for ACAT1. We have accordingly extended the discussion of ACAT inhibitors and added specific reference to these works in the section referred to.

6. 25-Hydroxycholesterol has been shown (JBC 250:4025 and JLR 40:2264) to drive plasma membrane cholesterol to the ER where it can be esterified by ACAT enzymes; this activity should be mentioned here, either as a complement or competitor to the ACAT allosteric activation theory proposed.

This is a very interesting adjunct to the molecular action of 25OHC. We have added mention (and citation) that 25OHC can drive cholesterol to the ER (however, this appears to require high concentrations of oxysterol*; one does wonder whether this mechanism is likely to contribute in vivo, although for completeness we have included note of this potential mechanism).

7. The statement is made on page 39, line 6 that sterol oxidation will modulate the formation of 25OHC; the basis for this conclusion is not clear.

We agree that this is too strong a statement. We have revised this as follows: 3- (or 7-) oxidation has potential to modulate the accumulation of cholesteryl esters in foam cells, although this remains to be demonstrated formally.

8. Page 41, line 17: some infectious agents home...
9. Page 43, line 19: by infection and... (not inflection)

We note both typos with thanks; these have been corrected.
Reviewer's report

Major Compulsory Revisions:
1) It is not clear why the authors use the definition of atherosclerotic (in fact 'arteriosclerotic' - author) vascular disease (summarized in the acronym ATH). This term is quite synonym of the most simple and well-known term atherosclerosis. They intend something of different from atherosclerosis?
2) Accordingly, to avoid confusion, some words detailing the differences between atherosclerosis and vascular disease (VaD or VD, cited only one time at page 6, line 2), a common pathology frequently overlapping AD but showing common features with AD, is missing.

The reviewer is right to raise this issue. The short answer is that 'athero' (from Greek meaning 'gruel') refers to a gruel-like deposit or swelling, but without specifying the location. Atheromas can be in any part of the body. For this reason we give also the more precise root 'arterio' (that specifies the arteries of the vasculature).

To clarify this point we have added a more detailed explanation to the definition of atherosclerosis at first mention in the text (and have removed the unnecessary definition from the abstract). We have also added a statement to the effect that, we believe, subclassification of AD according to vascular involvement needs to be revisited.

Minor Essential Revisions:
1) Please report through the text the full gene names followed by the acronyms and the genetic locus (this last between round brackets) according to the Human Gene Nomenclature Committee.

We thank the reviewer for this suggestion. For all human genes we now, at first mention, refer to the full gene name, followed by the official symbol, and the locus in round brackets.

2) I've noted only 23% of references that were published in the last three years (2011/2012/2013). Please refresh!

May we please try to convince the reviewer otherwise? In this work we have sought a comprehensive overview – including works that have become buried by the passage of time. In fact, an excellent example is given by the reviewer – 'with several interesting points (e.g. AD transmissibility)' – the original work on AD transmissibility in marmosets was published 20 years ago.

In another example, the stimulation of cholesterol esterification by 25OHC was discovered 40 years ago by Goldstein and Brown.

We believe strongly that in reviewing the field we have a duty to explore in depth and unearth key findings that are, today, now woefully overlooked. We hope the reviewer agrees with us.
3) At page 7, line 10, a reference for the polymorphisms associated with disordered lipid metabolism is missing.

We thank the reviewer for this suggestion. We have therefore added references to recent reviews and also to databases of AD and ATH genetics at this point in the text.

4) First three lines of the second paragraph at page 9. A reference for the genetics of the common APOE polymorphism is missing.

At the appropriate point in the text (in the following paragraph) we have now inserted citation of the most pertinent review papers addressing APOE polymorphisms.

5) At line 13, page 10, reference 50 is repeated.

We thank the reviewer for pointing out this error – this has been corrected.

Discretionary Revisions
1) In this review the authors reported a detailed description of the possible pathogenetic mechanisms underlying both Alzheimer’s diseases and atherosclerosis. The review is well written, with several interesting points (e.g. AD transmissibility), but although frequently verbose (e.g. AD infectious component). I think may a reduction of text length may improve the clarity of the concepts that authors wants review.

We are in two minds as to whether a significant reduction in text length would really help – because we would need to delete important material. Indeed, our colleagues have specifically applauded the fact that we have striven to cover (as far as practicable) all the pertinent data, and we would be very hesitant to compromise our inclusive approach. We beg forgiveness if, in this instance, we might disagree with the reviewer.

Other modifications/revisions
In addition to the above changes, several improvements have been made to the revised manuscript:

(i) Further typos have been corrected

(ii) Errors in the reference list have been corrected

We have also added mention of two highly relevant works

(iii) A systems biology approach looking at the differential transcriptome of AD brain centrally highlighted two functional modules – (a) Neurodegeneration/AD; (b) Cardiovascular Disease (Ray et al., 2008).

The authors concluded that these data ‘provide further support for the hypothesis that cardiovascular disease and Alzheimer’s disease are linked’. A short paragraph has been added to the revised manuscript.
(iv) Socia et al. (2010) have provided intriguing evidence that Abeta (implicated in both AD and ATH) might in fact be a defense mechanism – they describe antibacterial and antifungal properties of Abeta. We have added a short paragraph to this effect, with a perspective that clinical trials of Abeta removal have led to increased infection/encephalitis in treated patients. We have also added a further question to the Outstanding Questions Box concerning whether Abeta is a cause of disease or a defense mechanism.

Both reviewers have added significantly to the paper, and we thank them for their contributions (Acknowledgements).

(*In correspondence with Yvonne Lange, author of the key paper on ER redistribution driven by 25OHC, she advises that the effect is consistent at 25 microM, but increasingly inconsistent at 10 microM, whereas biological effects of 25OHC are seen at a 10-fold lower concentration).