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APP heterozygosity averts memory deficit in knock-in mice expressing the Danish Dementia BRI2 mutation

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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 18 March 2011

Thank you for submitting your manuscript to the EMBO Journal. Your study has now been seen by two referees and their comments are provided below.

As you can see, both referees appreciate the analysis and find it suitable for publication in the EMBO Journal pending adequate revisions. However more work is needed, referee #1 finds that further data is need to more precisely determine the effects of Bri2 on APP processing and in particular to look at Abeta42 levels. Should you be able to address the concerns raised then we would consider a revised manuscript. I should add that it is EMBO Journal policy to allow only a single round of revision, and acceptance of your manuscript will therefore depend on the completeness of your responses in this revised version. 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: http://www.nature.com/emboj/about/process.html

I thank you for the opportunity to consider your work for publication. I look forward to your revision.

Yours sincerely,

Editor
The EMBO Journal

REFEREE REPORTS

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Referee #1 (Remarks to the Author):

In the current manuscript D'Adamio and colleagues investigate whether APP is involved in the memory deficits observed in the mice with a Danish dementia BRI2 knock in mutation. In previous work the same authors have shown that Bri2 protein affects APP processing and that mutations in Bri2 change the overall spectrum of APP processing, enhancing its proteolytic cleavage by the different secretases.

The main observation in the current paper is that crossing the Bri2 knock in mice with APP haploinsufficient mice rescues LTP and learning and memory deficiencies. This is without any doubt interesting, as it suggests that the dementia observed in the Bri2 patients is induced by a similar APP mediated pathway as Alzheimer.

The manuscript needs however some further work. Several figures are not well displayed or are incomplete. This is discussed in further detail below. It would also be very important to determine more precisely the effects of Bri2 knock in mutation on APP processing. It must be possible to provide more definitive evidence on what APP metabolites are different in particular as the results pertain to the amyloid hypothesis of AD. I wonder whether it is possible to document these effects in homozygote knockin mice, in which case the effects on APP processing will be more significant and it might be possible to measure all Abeta species. In any event it should be possible to obtain some information on Abeta 42 levels.

Figure 1
-panel A controls should be included to show that the coprecipitation of APP and BRI2 is specific.
-What % of lysate material is used in the control lanes as compared to the IP lanes ?
- The purpose of panel B is unclear to me. Lettering should be consequent (T and R should be indicated in the IP lanes); this holds for the further panels as well.
-In panel E no explanation is given for the white bars. Overall more explanation in the figure legend is needed to make clear what is exactly done. I suppose that for instance the black bars in panel E reflect densitometric scans of blots stained with APP?

Figure 2
-Panel A: There is an enormous variation in APP salpha in wt; it is unclear whether the weak signals in lane 1 and 2 are the consequence of technical issues as in lanes 3 and 4 the signal is as strong as in FDDki. Therefore it is difficult to conclude from this experiment that there is less sAPPalpha.
-sAPP alpha quantitation is lacking in panel B
-the strong decrease in mature APP in panel E and F needs further discussion. Statistical marks are lacking in panel F.
-in panel I PS1 staining should be included as this is likely the most abundant secretase. Mr markers are lacking.
- the western blot going with panel J should be included to compare it with the blots in G.
The discussion section should discuss the differences with reports published by Jucker (Proc Natl Acad Sci U S A. 2010 Apr 27;107(17):7969-74. Epub 2010 Apr 12) and other reports that stress the importance of the amyloidosis caused by the Danish dementia mutation (J Neurochem. 2004 Jan;88(2):281-90). While I like the concept of knock in, and it avoids many potential artefacts of the over-used overexpression models, it remains important to put the findings of the current work in the broader context of what is published and to clarify the biases in the literature.

Referee #2 (Remarks to the Author):

The title is vague and I would suggest the title to be changed to "Heterozygosity of the APP gene prevents synaptic and memory impairment in knockin mice expressing the Danish Dementia mutation in BRI2".

The abstract should be more explicit, specifying the exact mouse models that are used in the current study (avoiding using vague terms such as a mouse model of danish dementia, etc.). More importantly, each of the major results shown in figures should be clearly described, so the readers will know exactly what type of analysis was performed and what were the results and final conclusions from each major experiments. The implication of the study should be minimally stated

in the abstract, and should be elaborated in the discussion.

The introduction should be focused on the background that is directly relevant to the current study, especially what is known about BRI2 and the authors' earlier publications.

Lastly, I would suggest the authors to include a model that summarizes this paper and their earlier findings, so that it will be easier for the readers to grasp what is the current understanding of Danish dementia associated with this BRI2 mutation.

In summary, this is a good paper and should be published in EMBO after the revision.

1st Revision - authors' response 01 April 2011

Referee #1

We thank the reviewer for his/her helpful suggestions, and we appreciate that the reviewer agrees that our findings "suggests that the dementia observed in the Bri2 patients is induced by a similar APP mediated pathway as Alzheimer".

Q1. *It would also be very important to determine more precisely the effects of Bri2 knock in mutation on APP processing. It must be possible to provide more definitive evidence on what APP metabolites are different in particular as the results pertain to the amyloid hypothesis of AD. I wonder whether it is possible to document these effects in homozygote knockin mice, in which case the effects on APP processing will be more significant and it might be possible to measure all Abeta species. In any event it should be possible to obtain some information on Abeta 42 levels.*

R1. The experiments shown in Figure 2 were performed using mice obtained from breeding FDD_{KI} females to FDD_{KI} males. From this breeding we attained both heterozygous and homozygous knock-in mice, in addition to wild type littermates. We did analyze all three genotypes but decided to show only the data relative to WT and heterozygous mutant mice, to adhere to the genetics of the human disease. To address the reviewer's request we now include the data for homozygous mutant mice. As shown in the new Figure 2, homozygous mutant mice have a greater increase in sAPPa and also show a statistically significant increase in sAPPb.

As for Ab measurements, in vivo clearance issues complicate measuring endogenous mouse CNS Ab. In addition, there is a technical hurdle that is unfortunately often ignored. Mouse brains give a colossal background signal in all Ab ELISA kits tested to date. This signals is seen in both WT and APP null mouse brains and conceals the specific Ab signals. The aspecific signal can be removed after further purification steps (Lanz & Schachter, 2006). Unfortunately these steps introduce a considerable variability due to the further manipulations. These two factors hamper the ability to detect in a statistically significant fashion changes in mouse Ab, unless they are massive. Thus, we analyzed MDFs isolated from FDD_{KI} mice crossed to human APP transgenic mice to measure human Abs in culture supernatants. MDFs with the Danish mutation in Bri2 produced significantly higher levels of both Ab40 and Ab42. These results are shown in Fig. 2G.

Q2. *Figure 1 -panel A controls should be included to show that the coprecipitation of APP and BRI2 is specific.*

R2. We apologize for the bad description of the experiment in the figure legend. The (–) sign indicates immunoprecipitations of samples with an irrelevant rabbit polyclonal antibody. Clearly mAPP is precipitated only by aBri2 (indicated with a + sign). In addition, we have previously shown that this antibody is capable of immuno-precipitating APP only from WT but not from Bri2 null mouse brains (Matsuda et al, 2008). Moreover, the evidence that the antibody precipitates mAPP and not imAPP further attests the specificity. An aspecific interaction would have probably not discriminated between these two APP species.

Q3. *Figure 1 -What % of lysate material is used in the control lanes as compared to the IP lanes?*

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R3. The lysates loaded in the control lanes represent 25% of the lysates originally used for the IP lanes. This information has been added to the legend of Fig. 1.

Q4. *Figure 1 - The purpose of panel B is unclear to me. Lettering should be consequent (T and R should be indicated in the IP lanes); this holds for the further panels as well.*

R4. The panel B is dispensable and it has been now deleted. We have indicated the IP lanes with T and R.

Q5. *Figure 1 -In panel E no explanation is given for the white bars. Overall more explanation in the figure legend is needed to make clear what is exactly done. I suppose that for instance the black bars in panel E reflect densitometric scans of blots stained with APP?*

R5. We apologize again for the bad description of the experiment in the figure legend. The requested information has been added and the description of the experiments is now much more detailed and should clearly explain the data.

Q6. *Figure 2 -Panel A: There is an enormous variation in APP salpha in wt; it is unclear whether the weak signals in lane 1 and 2 are the consequence of technical issues as in lanes 3 and 4 the signal is as strong as in FDDki. Therefore it is difficult to conclude from this experiment that there is less sAPPalpha.*

R6. This variation can happen when analyzing endogenous soluble APP fragments. However, the data overall (including the new data for FDD_{KI} homozygous brains and MDFs) strongly support the claim that sAPPa levels are significantly increased in FDD_{KI} mice.

Q7. *Figure 2 -sAPP alpha quantitation is lacking in panel B*

R7. We apologize for the mistake. The data are now included in Fig. 2D.

Q8. *Figure 2 -the strong decrease in mature APP in panel E and F needs further discussion. Statistical marks are lacking in panel F.*

R8. We now discuss the decrease in APP in Figure 2 legends as follows: "It is worth noting that the levels of full length APP decreased in knock-in MDFs. This is consistent with an increase in cleavage of APP by a-secretase in these cells."

Statistical marks are now included in Fig. 2D.

Q9. *Figure 2 -in panel I PS1 staining should be included as this is likely the most abundant secretase. Mr markers are lacking.*

R9. The PS1 WB and the Markers are now included.

Q10. *Figure 2 - the western blot going with panel J should be included to compare it with the blots in G.*

R10. The western blot for ALID1 is included in Fig. 2K.

Q11. *The discussion section should discuss the differences with reports published by Jucker (Proc Natl Acad Sci U S A. 2010 Apr 27;107(17):7969-74. Epub 2010 Apr 12) and other reports that stress the importance of the amyloidosis caused by the Danish dementia mutation (J Neurochem. 2004 Jan;88(2):281-90). While I like the concept of knock in, and it avoids many potential artefacts of the over-used overexpression models, it remains important to put the findings of the current work in the broader context of what is published and to clarify the biases in the literature.*

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R11. Both papers are now discussed in the discussion section of the revised manuscript. The first paper is discussed as follows: "In contrast with the amyloid cascade hypothesis (Hardy & Selkoe, 2002), our data present no evidence supporting a role for ADan in synaptic plasticity and memory deficits. Transgenic mouse models of FDD reproduce amyloidosis (Coomaraswamy et al, 2010; Vidal et al, 2009). These mice are genetically non-congruous with the human diseases since the mutant transgene is expressed in an artificial quantitative-spatio-temporal manner, and do not replicate loss of function, given the persistence of the two endogenous WT mouse alleles. On the contrary, FDD transgenic mice express elevated brain levels of mBRI2 (Coomaraswamy et al, 2010) which is opposite to what observed in FDD_{KI} mice and, more importantly, in FDD human cases (Tamayev et al, 2010). Despite the considerable amyloidosis, memory loss has not been described in FDD transgenic mice (Coomaraswamy et al, 2010; Vidal et al, 2009). It is also worth noting that in human FDD cases ADan and Ab42co-deposits in a subset of amyloid plaques (Vidal et al, 2000). However, the expression of the FDD transgene in human APP transgenic mice not only does not lead to formation of ADan/Ab42 mixed plaques, but in contradiction causes an extensive reduction in Ab amyloid plaques (Coomaraswamy et al, 2010). This is consistent with the hypothesis that an increase in mBRI2 levels reduces processing of APP (Matsuda et al, 2008). Thus, the lack of co-deposition of human ADan and Ab42 and the inhibitory effect on Ab42 in FDD transgenic mice (Coomaraswamy et al, 2010) further stresses the incongruence of the transgenic model with the human disease."

We comment the second manuscript as follows: **"**The ADan peptide may start out other clinical symptoms that are present in Danish Dementia patients, such as cataracts, deafness and progressive ataxia, but are not replicated in FDD_{KI} mice. In addition, in late phase of the disease accumulation of oligomeric ADan, which is toxic to neuronal cell lines (Gibson et al, 2004) may cause neuronal degeneration and neuronal loss which are associated with FDD but are not obviously detectable in FDD_{KI} mice. The neurotoxicity of oligomeric ADan may also be limited to in vitro systems since ADan amyloidosis does not lead to neuronal loss in transgenic mice (Coomaraswamy et al, 2010; Vidal et al, 2009)."

Referee #2

We thank the reviewer for his/her helpful suggestions, and for considering the work worth of publication in the EMBO Journal.

Q1. *The title is vague and I would suggest the title to be changed to "Heterozygosity of the APP gene prevents synaptic and memory impairment in knockin mice expressing the Danish Dementia mutation in BRI2".*

R1. We have changed the title according to the excellent reviewer's suggestion. However, the suggested title was too long (more than 100 characters). Therefore, we had to modify it slightly. The current title is: "**APP heterozygosity averts memory deficit in knock-in mice expressing the Danish Dementia BRI2 mutant"**

Q2. *The abstract should be more explicit, specifying the exact mouse models that are used in the current study (avoiding using vague terms such as a mouse model of danish dementia, etc.). More importantly, each of the major results shown in figures should be clearly described, so the readers will know exactly what type of analysis was performed and what were the results and final conclusions from each major experiments. The implication of the study should be minimally stated in the abstract, and should be elaborated in the discussion.*

R2. We agree that the abstract was too conceptual. The new version describes the data more accurately and in detail and reserves only the last sentence to the interpretation of the significance.

Q3. *The introduction should be focused on the background that is directly relevant to the current study, especially what is known about BRI2 and the authors' earlier publications.*

R3. We have modified the introduction following the suggestions of the reviewer.

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Q4. *Lastly, I would suggest the authors to include a model that summarizes this paper and their earlier findings, so that it will be easier for the readers to grasp what is the current understanding of Danish dementia associated with this BRI2 mutation.*

R4. We have revised Fig. 6, which now includes a comparison between the Amyloid and the Loss of function models in addition to three models that depict how loss of BRI2 function and consequent changes in APP signaling can lead to memory loss.

Coomaraswamy J, Kilger E, Wolfing H, Schafer C, Kaeser SA, Wegenast-Braun BM, Hefendehl JK, Wolburg H, Mazzella M, Ghiso J, Goedert M, Akiyama H, Garcia-Sierra F, Wolfer DP, Mathews PM, Jucker M (2010) Modeling familial Danish dementia in mice supports the concept of the amyloid hypothesis of Alzheimer's disease. Proc Natl Acad Sci U S A **107**(17)**:** 7969-7974

Gibson G, Gunasekera N, Lee M, Lelyveld V, El-Agnaf OM, Wright A, Austen B (2004) Oligomerization and neurotoxicity of the amyloid ADan peptide implicated in familial Danish dementia. J Neurochem **88**(2)**:** 281-290

Hardy J, Selkoe DJ (2002) The amyloid hypothesis of Alzheimer's disease: progress and problems on the road to therapeutics. Science **297**(5580)**:** 353-356

Lanz TA, Schachter JB (2006) Demonstration of a common artifact in immunosorbent assays of brain extracts: development of a solid-phase extraction protocol to enable measurement of amyloid-beta from wild-type rodent brain. J Neurosci Methods **157**(1)**:** 71-81

Matsuda S, Giliberto L, Matsuda Y, McGowan EM, D'Adamio L (2008) BRI2 inhibits amyloid beta-peptide precursor protein processing by interfering with the docking of secretases to the substrate. J Neurosci **28**(35)**:** 8668-8676

Tamayev R, Matsuda S, Fa M, Arancio O, D'Adamio L (2010) Danish dementia mice suggest that loss of function and not the amyloid cascade causes synaptic plasticity and memory deficits. Proc Natl Acad Sci U S A **107**(48)**:** 20822-20827

Vidal R, Barbeito AG, Miravalle L, Ghetti B (2009) Cerebral amyloid angiopathy and parenchymal amyloid deposition in transgenic mice expressing the Danish mutant form of human BRI2. Brain Pathol **19**(1)**:** 58-68

Vidal R, Revesz T, Rostagno A, Kim E, Holton JL, Bek T, Bojsen-Moller M, Braendgaard H, Plant G, Ghiso J, Frangione B (2000) A decamer duplication in the 3' region of the BRI gene originates an amyloid peptide that is associated with dementia in a Danish kindred. Proc Natl Acad Sci U S A **97**(9)**:** 4920-4925

2nd Editorial Decision 27 April 2011

Thank you for submitting your revised manuscript to the EMBO Journal. I asked the original referee # 1 to review the revised version and I have now received the comments. As you can see below, the referee appreciates the introduced changes and supports publication in the EMBO Journal. The referee has one remaining text suggestion - see below - that I would like to ask you to incorporate in

the final version. You can send us an amended PDF file by email and we will upload it for you. Once we receive the file we will proceed with the acceptance of the paper for publication here.

Editor
The EMBO journal

REFEREE REPORT

Referee #1

The authors have constructively responded to my criticism. I have a minor remark, ie in the introduction it is written:
"an intracellular product termed the APP Intracellular Domain (AID or AICD) that regulates cell death (Passer et al, 2000) and gene transcription (Cao & Sudhof, 2001)." The role of AICD or AID as the authors prefer to call it, is not firmly established and there are probably more negative reports on the role of AICD in gene regulation than confirmations. This might be mentioned.