Amyloid Precursor Protein mutation E682K at the alternative β-secretase cleavage β'-site increases Aβ generation

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Review timeline:

<table>
<thead>
<tr>
<th>Event</th>
<th>Date</th>
</tr>
</thead>
<tbody>
<tr>
<td>Submission date</td>
<td>20 December 2010</td>
</tr>
<tr>
<td>Editorial Decision</td>
<td>25 January 2011</td>
</tr>
<tr>
<td>Revision received</td>
<td>04 February 2011</td>
</tr>
<tr>
<td>Editorial Decision</td>
<td>06 March 2011</td>
</tr>
<tr>
<td>Accepted</td>
<td>06 March 2011</td>
</tr>
</tbody>
</table>

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

1st Editorial Decision 25 January 2011

Thank you for the submission of your manuscript "A novel APP mutation E682K at the alternative β-secretase cleavage site (β'-site) causes increased Aβ generation and is associated with Alzheimer disease". We have now heard back from the three referees whom we asked to evaluate your manuscript. You will see that they find the topic of your manuscript potentially interesting. However, they also raise some concerns on the study, which should be addressed in a revision of the manuscript.

In particular, reviewer 2 and 3 highlight that it should be investigated whether the asymptomatic brother of the patient has sub-clinical signs of AD. Of note, reviewer 1 feels that the claims regarding the genetic association of the mutation with AD should be moderated and the title of the study amended accordingly. On a more editorial note, we would also recommend to shorten the title somewhat to improve clarity (eg avoid parenthesis).

Given the positive evaluations, we would like to welcome a revision of your manuscript if you can convincingly address the issues that have been raised within the space and time constraints outlined below.

Revised manuscripts should be submitted within three months of a request for revision. They will otherwise be treated as new submissions, unless discussed otherwise with the editor.

I look forward to seeing a revised form of your manuscript as soon as possible.

Yours sincerely,

Editor
EMBO Molecular Medicine
REFEREE REPORTS:

Referee #1:

The only problem with this manuscript is that the genetic proof of pathogenicity is not convincing: there is nothing that can be done about this. All the samples that are available to the authors have been assessed... but they may be wrong and this needs to be acknowledged more prominently even in the title (strictly, the variant has not been shown to be associated with AD because one should always ignore the first case). Guerreiro has recently suggested a mutation classification scheme for "Alzheimer pathogenecity" which the authors may wish to use in this discussion.

But, if the mutation is pathogenic, clearly this manuscript will be of interest.

Referee #2:

It was a pleasure to read the manuscript by Zhou and colleagues as it is novel, informative and well written. The authors found a new APP mutation, E682K, and analyzed its phenotype by molecular cell biological means. The mutation caused overproduction of Abeta and an increase in the ratio of Abeta42/Abeta40. It is also an interesting discovery that beta' cleavage of APP takes place under physiological conditions. These findings deserve attention by a large number of readers.

Major criticisms

1. Although this is not a prerequisite, it would strengthen and make the study more convincing, if the patient's brother, a silent mutation carrier, can be analyzed by PIB-PET to clarify whether he is in a presymptomatic stage of AD.
2. It is not clear how the mutatnt Abeta was quantified by ELISA. Were the synthetic mutant peptides used for calibration? They should be because the affinity for the capture antibody may be different between the wild-type and mutant peptides. This could lead to different results and conclusions.

A minor note

1. In the 5th paragraph of DISCUSSION, "a significantly effect" should be changed to "a significant effect."

Referee #3:

The Authors found a novel mutation at codon 682 of the APP gene (i.e., E682K) in a patient with early onset Alzheimer's disease, and expressed this mutation in mouse primary cortical neurons and CHO cells to evaluate its functional effects on APP processing, and Ab aggregation and toxicity. The study showed that the E682K mutation, which is located at the b' BACE1-cleavage site within the Ab sequence, blocks b' processing and enhances b-site cleavage of APP, resulting in two- to three-fold increase in C99, sAPPb and total Ab levels. Furthermore, the mutation was found to modulate gamma-secretase activity leading to slight but significant increase in Ab42/Ab40 ratio. Conversely, the E682K mutation did not affect substantially the aggregation kinetics and neurotoxicity of synthetic Ab1-42 peptides.

This work provides important new information to the field of Alzheimer's disease. First, the Authors present strong evidence that cleavage at the b'-site by BACE1 is quantitatively more important for normal APP metabolism than previously thought, as this pathway generates about 25% of the major APP C-terminal fragments. Second, blocking this pathway - as occurs with the E682K mutation - shifts APP cleavage by BACE1 from b'- to b-site, leading to increased production of full-length Ab and the development of early-onset Alzheimer's disease. Thus the b' cleavage seems to be protective rather than a pathway that contributes to AD pathogenesis as suggested previously. Inhibition of BACE1 is one of the most attractive strategies for AD therapeutics. The data presented here should stimulate the identification and development of compounds with differential inhibitory activity on the b- and b'-site.
The experimental plan was well designed and the work was carried out thoroughly with appropriate controls. The data are reported with clarity and documented with figures of good quality.

A critical point is that the brother of the patient is clinically unremarkable at the age of 53 (the index case developed initial symptoms at the age of 47) although he carries the E682K mutation. It would be important to verify whether this asymptomatic brother has amyloid deposits in the brain using PIB-imaging. Furthermore, it would be valuable if the in vitro data could be confirmed on fibroblasts from the patient and his brother. These data would strongly support the pathogenic role of the E683K mutation.

The reference to Fig. 2B at page 4, line 13, is not correct and should be changed to Fig. 3B.

Referee #1:

The only problem with this manuscript is that the genetic proof of pathogenicity is not convincing: there is nothing that can be done about this. All the samples that are available to the authors have been assessed... but they may be wrong and this needs to be acknowledged more prominently even in the title (strictly, the variant has not been shown to be associated with AD because one should always ignore the first case). Guerreiro has recently suggested a mutation classification scheme for "Alzheimer pathogenecity" which the authors may wish to use in this discussion.

As suggested by the referee # 1, we included the mutation classification scheme from (Guerreiro et al, 2010) in the discussion. According to the proposed scheme, the APP E682K mutation described in this work is assigned as “probably pathogenic”, since it is found in one case of an early-onset AD, absent from 940 healthy controls, and affects Aβ generation.

It should be also mentioned that this is a novel mutation site on APP (according to the scheme, the pathogenecity gets a “promotion” to a higher category of disease risk when the new mutation hits a reported site with known disease-causing mutations). The current mutation however hits a major APP processing site by β-secretase, and it affects the Aβ generation to a similar extend as a reported disease causing ‘Flemish’ mutation. We believe our functional assay data strongly support the probable pathogenecity of the novel mutation and this has taught us important insights in the functional importance of the β'-site cleavage by BACE1.

Referee #2:

Major criticisms

1. Although this is not a prerequisite, it would strengthen and make the study more convincing, if the patient's brother, a silent mutation carrier, can be analyzed by PIB-PET to clarify whether he is in a presymptomatic stage of AD.

We agree with the referee that PIB-PET scan of the brother would be a way to clarify whether he is in a presymptomatic stage of AD and strengthen the manuscript. However, we currently cannot perform the PET scan due to ethical issues: amyloid PET is so far not an approved protocol for the asymptomatic carrier, and he is currently unaware of his status. The study protocol does not allow to contact the carrier again as this would inform him implicitly. As also discussed with referee 1 we have now more explicitly clarified the lack of genetic proof for the causality of the mutation and have used a recent published paper to discuss the classification as “probably pathogenic”. We have to wait for other cases to eventually promote the mutation to a more definitive category. However, as discussed in the manuscript, this work mainly focused on functional analysis to clarify the probable pathogenecity of the novel mutation and this has taught us important insights in the functional importance of the β'-site cleavage by BACE1.
2. It is not clear how the mutant Abeta was quantified by ELISA. Were the synthetic mutant peptides used for calibration? They should be because the affinity for the capture antibody may be different between the wild-type and mutant peptides. This could lead to different results and conclusions.

We quantified Aβ from conditioned medium by end-specific ELISAs. As described in material and methods, monoclonal antibody JRF/cAβ40/28 or JRF/cAβ42/26 (Mathews et al, 2002), raised against the C-terminal last five or ten residues of Aβ species terminating at 40 or 42, were used as capturing antibodies. HRP-conjugated JRF/AβN/25 (Mathews et al, 2002), raised against the N-terminal first 7 amino acids (requires the β-site cleavage) of human Aβ was used as detecting antibody for full length Aβ. Synthetic WT Aβ peptide was used to establish standard curves. Since the mutation E682K (E11K of Aβ sequence) is distant from the C-terminus of Aβ, it is unlikely to affect the affinity of the C-terminal antibodies JRF/cAβ40/28 or JRF/cAβ42/26 for the mutant peptide. To prove however that the affinity of the N-terminal antibody JRF/AβN/25 for Aβ peptide was not affected by the E682K (E11K mutation), we conducted a competition ELISA to determine the affinities of this antibody for WT and mutant peptide. As shown in the novel supplemental figure 3, no difference in the affinities was observed. We thus conclude that this mutation does not affect the binding of the N-terminal antibody to the mutant Aβ peptide.

It should also be mentioned that the results from the Aβ ELISA measurements is in agreement with the results from other APP fragments detected by Western blots using different antibodies, i.e. sAPPβ and CTFβ, which show similar fold increases. Moreover, when we immunoprecipitate Aβ using only C-terminal antibodies JRF/cAβ40/28 and JRF/cAβ42/26 in metabolic labeling assays (Fig 3D), we also observed similar fold increases in the generation of mutant Aβ peptide. We thus conclude that the end-specific Aβ ELISA was giving an unbiased report on the WT and mutant Aβ levels.

Referee #3:

A critical point is that the brother of the patient is clinically unremarkable at the age of 53 (the index case developed initial symptoms at the age of 47) although he carries the E682K mutation. It would be important to verify whether this asymptomatic brother has amyloid deposits in the brain using PIB-imaging. Furthermore, it would be valuable if the in vitro data could be confirmed on fibroblasts from the patient and his brother. These data would strongly support the pathogenic role of the E683K mutation.

Please refer to the answer to referee #2. We entirely agree that PIB-PET scan of the brother would strengthen the manuscript; however, unfortunately, we currently cannot perform the amyloid PET scan due to study design and ethical issues. The asymptomatic brother is not aware of his status (carrying the mutation), and we are not approved to contact him again, neither for brain scan nor to obtain fibroblasts. However, we were allowed to obtain fibroblasts from the index patient, and we performed experiments with the fibroblasts. The reason we did not incorporate the fibroblast data in the current manuscript is the huge variation in Aβ levels in the control cell lines. We believe that this variation partially reflects the big variations in the growth of the different fibroblast samples we were working with. We add part of those data to this letter. We analyzed the APP processing in the patient’s fibroblasts and in control fibroblasts (obtained from four unrelated individuals). We first detected the C-terminal fragments of APP from cell extracts by immunoprecipitation and Western blot using a pAb B63 against the C-terminus of APP. From independent experiments, we observed a significant increase in the generation of C99 from the patient’s fibroblasts as compared to control fibroblasts. It should be noted that we applied in this experiment the same amount of total protein from different fibroblasts in immunoprecipitation, thus the growth speed of fibroblasts had minimal effects on the steady state APP CTFs.

However, when we turned to analyze the secreted Aβ (by ELISA) from 48 hours conditioned media, we had big variation in the Aβ levels from different fibroblast samples as determined by independent experiments (not shown). As detected by western blots, the levels of sAPPtotal from conditioned media also had big variation. During the experiments, we observed that the human fibroblasts we used grew at very different speeds, and growth varied a lot even within one cell line, in function of the cell passage.
To sum up, the data from APP processing at steady state (significant increase in C99 level) is clearly in agreement with the data obtained from neuronal/CHO cells cultures, whereas the fibroblast data with regard to Aβ levels was not conclusive due to the big variation in the obtained results. We therefore prefer not to include these data in the manuscript.

2nd Editorial Decision 06 March 2011

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Congratulations on your interesting work and best wishes,

Editor
EMBO Molecular Medicine

REFEREE REPORTS

Referee #1:

The authors have done all that was possible for them to do and have modified their discussion to be more sensitive to the possibilities that the mutation is not pathogenic

Referee #2 Remarks on Novelty/Model system:

In ethical terms, it is relevant not to examine the patient's brother, a silent mutation carrier, any further.

Referee #2:

This is a case in which there is a conflict ethics and scientific perfectionism. Without doubt, ethics should be given top priority. Besides, the study provides a conceptual advancement about the beta-secretase activity. This reviewer strongly believes that the paper should be published in the present form.

Referee #3:

Due to ethical issues, the Authors could not perform the suggested studies - i.e., PIB-PET and analysis of fibroblasts - on the asymptomatic carrier of the E682K mutation, to support the hypothesis that this mutation is really pathogenic. For this reason, in the revised version of the manuscript they classified the mutation as "probably pathogenic" and acknowledged that further studies on additional cases are necessary to grade this genetic variant as "definitely pathogenic".

Nevertheless, I agree with the Authors that this work provides important and novel insight in the biological significance of the β-site processing of APP in physiological conditions and disease.