EMBO Molecular Medicine

An Alzheimer associated TREM2 variant occurs at the ADAM cleavage site and affects shedding and phagocytic function

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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.)

Editor: Céline Carret

1st Editorial Decision 15 March 2017

Thank you for the submission of your manuscript to EMBO Molecular Medicine. We have now heard back from the three referees who we asked to evaluate your manuscript.

You will see that all 3 are enthusiastic about the data and are supportive of publication pending minor revisions. We also would like to raise your attention to some needed editorial amendments.

1) Pleas address the minor comments of all referees. Please provide a letter INCLUDING the reviewer's reports and your detailed responses to their comments (as Word file).

2) Source Data:
As you know, we now encourage the publication of source data, particularly for electrophoretic gels, blots, but also microscopy images with the aim of making primary data more accessible and transparent to the reader. Would you be willing to provide a PDF file per figure that contains the original, uncropped and unprocessed scans of all or key gels used in the figure? The PDF files should be labeled with the appropriate figure/panel number (1 file/figure), and should have molecular weight markers; further annotation may be useful but is not essential. The PDF files will be published online with the article as supplementary "Source Data" files.

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

***** Reviewer's comments *****

Referee #1 (Remarks):

In this manuscript by Schlepckowet al., the authors investigate the effects of TREM2 variants associated with Alzheimer's disease. The authors found that the H157Y mutation increases shedding of mutant TREM2, and reduced phagocytic activity. The authors wrote a clear introduction, and performed their experiments rigorously. Therefore, this research manuscript is suitable for publication in EMBO after addressing the following comments:

Minor comments
1. The authors did not clarify why TREM2 p.H157Y leads to enhanced degradation of CTF by gamma-secretase cleavage.
2. How does the phagocytic function of WT TREM2 compare to mutant TREM2 (p.H157Y) in vivo? Has this been investigated by the authors or other groups? This can be discussed to address of the biological consequences.
3. Figure legends for figures 1-3 should include description of all abbreviations used in the figure
4. Fig.1C, E, H, K should specify in the legend or Figure what the two lanes for each condition represent. Do the two lanes represent independent experiments?
5. Fig.1K: in the upper panel, sTREM2 in both lanes of TREM2-TEVFlag are not on the same height on the blot. Please clarify.

Referee #2 (Remarks):

The manuscript by Schlepckow et al. presents a careful characterization of the effect of different mutations in Triggering Receptor expressed on Myeloid Cells 2 (TREM2) on its surface levels and ability to function as a receptor involved in phagocytosis. The authors characterize the cleavage site of TREM2 for ADAM10 and ADAM17, finding it to be identical for these two principal sheddases. Moreover, they compare and contrast the effect of a mutation in the Ig-like domain of TREM2 (T66M) on its cell surface expression with that of a mutation at the cleavage site (H157Y) that has been implicated in Alzheimer's disease. Interestingly, both types of mutations clearly decrease surface levels of full length TREM2, but by very different mechanisms. The mutation in the Ig-like domain lead to intracellular retention, whereas the H157Y mutation close to the cleavage site increases shedding, thus also lowering cell surface levels. The authors employ a phagocytosis assay with labeled E. coli to provide functional evidence that the H157Y mutation lower phagocytosis, suggesting that this mutation could also affect phagocytosis of Abeta plaques. Thus, they provide a compelling explanation for how the H157Y mutation might contribute to AD.

Overall, the data are of the highest quality, with excellent Western blots and mass spectrometric data and a convincing experimental approach. The analysis of E. coli endocytosis rounds out the study in a way that points to the functional relevance of the H157Y mutation. The introduction is well written and provides a good overview of the current status of the field, including previous work implicating ADAM10 as a key sheddase for TREM2. The discussion is supported by the results, and provides an appropriately balanced interpretation of these interesting findings. This is a timely contribution to the understanding of the role of TREM2 in AD, which should be of significant interest to the readers of EMBO MM.

Critique:
This reviewer has no concerns regarding the data or their interpretation, but feels that this already excellent manuscript could be further improved by performing the phagocytosis assay shown in figure 3 with the T66M mutant in the Ig-like domain to provide a side-by-side comparison. The outcome seems quite predictable, but would nevertheless be interesting to include.

Referee #3 (Comments on Novelty/Model System):

The present manuscript describes the identification of the cleavage site of TREM2, which very interestingly, corresponds the location of a known pathogenic mutant, p.H157Y. This finding is highly novel as it represents the first report to identify the TREM2 cleavage site. Given the known

impact of this and other TREM2 mutations in the pathogenesis of 'sporadic' AD, this information is timely and highly medically relevant.

The authors were thorough in their methodology used to definitely determine the cleavage site, employing multiple strategies to confirm their finding. Similarly, they utilized multiple cell systems to bolster the adequacy of their model system, which is appreciated.

Referee #3 (Remarks):

This manuscript serves as an important addition to previous findings from this group relating to TREM2 processing and the impact of pathogenic mutations on TREM2 processing and cell function.

The manuscript is clearly written and likewise, data are clearly presented in the corresponding figures. Methods and statistics are sufficiently described and experimental rigor is also sufficient in that multiple replicates are represented in the figures.

For all of the above reasons, this manuscript is suitable for publication with consideration to the following minor revision:

The authors end the manuscript with a demonstration of functional effects of the p.H157Y mutation on TREM2 relating specifically to phagocytosis. While the attempt to demonstrate the functional consequences of this mutation is appreciated, and this reviewer does not feel that functional experiment is necessary for publication, the experiment presented fails to sufficiently address the question asked. If included in the manuscript, the following considerations should be addressed:

Microglia cells are highly influenced by micro-environmental conditions and their functional/ phagocytic response is highly context and stimulus specific. For that reason, one should take care when extrapolating in vitro findings from cell lines to the in vivo setting. Along those lines, it is important to mimic relevant conditions as much as possible. In this case, phagocytosis of Abeta would be much more relevant and appropriate than E coli and should be included since phagocytosis of E coli may not employ the same phagocytic mechanisms as Abeta. Abeta phagocytosis has been demonstrated by this group in past experiments, demonstrating their ability to conduct such an experiment.

Furthermore, microglial phagocytosis of amyloid plaques in the human AD brain (in the absence of additional stimulation, e.g. vaccination) is highly controversial and poorly demonstrated and understood thus far, both in terms of the occurrence and impact on disease pathogenesis. For that reason, the significance of the functional (phagocytosis) data presented (in p.H157Y mutants) on the pathogenesis of AD should be carefully interpreted and described given the high level of public interest in this topic to responsibly ensure appropriate understanding of these findings by non-specialists.

Overall, this is a very nice paper that I believe EMOBO readers will appreciate and from which they will derive benefit.

1st Revision - authors' response 26 June 2017

Referee #1
The authors did not clarify why TREM2 p.H157Y leads to enhanced degradation of CTF by gamma-secretase cleavage.

We agree with the referee that this is a major open question, specifically since in the accompanying paper the CTF was readily detected. When addressing this point we thought that TREM2 always forms a tight complex with DAP12 in myeloid cells. However, in HEK293, which we used to express ectopic TREM2 no DAP12 is expressed. Dimer formation is likely to abolish gamma-secretase from cleaving, since its substrate binding site as well as its catalytically active site apparently accepts monomeric substrates only. Thus, TREM2/DAP12 dimer formation may lead to the inhibition of gamma-secretase mediated clearance of TREM2 and therefore to stabilization of

TREM2 CTFs. Upon co-expression of TREM2 and DAP12 we indeed found a robust stabilization of TREM2 CTFs including the TREM2 H157Y CTF. This is now shown in the new figure 2I. Moreover, since TREM2 H157Y is a better substrate for ADAM cleavage, we assume in analogy to Swedish mutant APP (Haass et al., Nature Medicine, 1995), that monomeric TREM2 H157Y is cleaved earlier during the transport through the secretory pathway and then directly targeted to endosomes/lysosomes. This is now discussed accordingly.

How does the phagocytic function of WT TREM2 compare to mutant TREM2 (p.H157Y) in vivo? Has this been investigated by the authors or other groups? This can be discussed to address of the biological consequences.

We currently do not know this. However, in our recent amyloid seeding experiments in the TREM2 KO and the TREM2 T66M mouse, we observed enhanced seeding and higher coverage of the amyloid plaque area indicating a TREM2 dependent phagocytic clearance of amyloid plaques. Unfortunately, the H157Y mutation has not yet been introduced into the mouse genome. Nevertheless, so far we see a good correlation between in vitro and in vivo phenotypes of the T66M mutation (see also our recent publication by Kleinberger et al., EMBO J, 2017) and would expect similar parallels in H157Y mice. This is now discussed accordingly.

Figure legends for figures 1-3 should include description of all abbreviations used in the figure.

Abbreviations are explained in the main text, when used the first time.

Fig.1C, E, H, K should specify in the legend or Figure what the two lanes for each condition represent. Do the two lanes represent independent experiments?

The two lanes represent samples from two separate wells seeded at the same time. This is now mentioned in the Fig. 1 legend.

Fig.1K: in the upper panel, sTREM2 in both lanes of TREM2-TEVFlag are not on the same height on the blot. Please clarify.

The supernatants of cultured cells contain large amounts of serum proteins, which slightly affect protein migration. Variability in the apparent migration pattern are therefore not due to different molecular weights.

Referee #2

This reviewer has no concerns regarding the data or their interpretation, but feels that this already excellent manuscript could be further improved by performing the phagocytosis assay shown in figure 3 with the T66M mutant in the Ig-like domain to provide a side-by-side comparison.

We showed in our two previous publications that TREM2 T66M inhibits phagocytosis. We first demonstrated that the T66M mutation impairs phagocytosis in HEK293 overexpressing T66M mutant TREM2 (Kleinberger et al., Science Translational Medicine, 2014). More recently we confirmed this finding in mice where we introduced the T66M mutation via CRISPR/Cas9 genome editing (Kleinberger et al., EMBO J, 2017). Since T66M is an almost complete loss of function mutation, the effects on phagocytosis are rather pronounced.

Referee #3

For that reason, one should take care when extrapolating in vitro findings from cell lines to the in vivo setting. Along those lines, it is important to mimic relevant conditions as much as possible. In this case, phagocytosis of Abeta would be much more relevant and appropriate than E coli and should be included since phagocytosis of E coli may not employ the same phagocytic mechanisms as Abeta.

For in vivo analysis one must introduce the H157Y mutation into the mouse genome. We are about to do this, however, generation and phenotypic analyses of this mouse mutant will take a substantial amount of time and is therefore part of a different project. However, we want to emphasize that in

our recent publication (Kleinberger et al., EMBO J, 2017) we demonstrated a reduction of Abeta uptake in bone marrow derived macrophages carrying the T66M mutation. We are now pointing out in the discussion that functional implications of our study must be confirmed in an in vivo setting, at best in a CRISPR/Cas9 edited mouse model expressing endogenous TREM2 H157Y.

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YOU MUST COMPLETE ALL CELLS WITH A PINK BACKGROUND ↓

PLEASE NOTE THAT THIS CHECKLIST WILL BE PUBLISHED ALONGSIDE YOUR PAPER

Corresponding Author Name: Christian Haass
Journal Submitted to: EMBO Molecular Medicine
Manuscript Number: EMM-2017-07672

Reporting Checklist For Life Sciences Articles (Rev. July 2015)

This checklist is used to ensure good reporting standards and to improve the reproducibility of published results. These guidelines are consistent with the Principles and Guidelines for Reporting Preclinical Research issued by the NIH in 2014. Please follow the journal's authorship guidelines in preparing your manuscript.

A- 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.
- figure panels include only data points, measurements or observations that can be compared to each other in a scientifically meaningful way.
- graphs 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 and any statistical test employed should be justified
- Source Data should be included to report the data underlying graphs. Please follow the guidelines set out in the author ship 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 $\chi 2$ 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?
  - exact statistical test results, e.g., P values = x but not P values < x;
  - definition of 'center values' as median or average;
  - definition of error bars as s.d. or s.e.m.

Any descriptions too long for the figure legend should be included in the methods section and/or with the source data.

Please ensure that the answers to the following questions are reported in the manuscript itself. We encourage you to include a specific subsection in the methods section for statistics, reagents, animal models and human subjects.

In the pink boxes below, provide the page number(s) of the manuscript draft or figure legend(s) where the information can be located. Every question should be answered. If the question is not relevant to your research, please write NA (non applicable).

B- Statistics and general methods

Please fill out these boxes ↓ (Do not worry if you cannot see all your text once you press return)

1.a. How was the sample size chosen to ensure adequate power to detect a pre-specified effect size?	Sample size was chosen according to our experience with in vitro assays, cell biological and biochemical experiments.
1.b. For animal studies, include a statement about sample size estimate even if no statistical methods were used.	NA
2. Describe inclusion/exclusion criteria if samples or animals were excluded from the analysis. Were the criteria pre-established?	NA
3. Were any steps taken to minimize the effects of subjective bias when allocating animals/samples to treatment (e.g. randomization procedure)? If yes, please describe.	No.
For animal studies, include a statement about randomization even if no randomization was used.	NA
4.a. Were any steps taken to minimize the effects of subjective bias during group allocation or/and when assessing results (e.g. blinding of the investigator)? If yes please describe.	No.
4.b. For animal studies, include a statement about blinding even if no blinding was done	NA
5. For every figure, are statistical tests justified as appropriate?	Yes. Every statistical test is appropriate and it is described in each figure and in the statistics section in the Materials and Methods part of the paper.
Do the data meet the assumptions of the tests (e.g., normal distribution)? Describe any methods used to assess it.	Normal distributions are assumed. The small sample sizes per condition used in this study do not allow valid assessment of the distribution. The samples did not contain any outlier that may bias our results.
Is there an estimate of variation within each group of data?	Yes. The SEM intervals of all groups compared are shown in the graphs.
Is the variance similar between the groups that are being statistically compared?	The variances were similar between the experimental groups compared.

C- Reagents

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6. To show that antibodies were profiled for use in the system under study (assay and species), provide a citation, catalog number and/or clone number, supplementary information or reference to an antibody validation profile. e.g., Antibodypedia (see link list at top right), 1DegreeBio (see link list at top right).	The antibody specific for the C terminus of human TREM2 (clone 9D11) was generated by Dr. Regina Feederle (Helmholtz Center Munich). Details are given in the Materials and Methods section. All other antibodies are commercially available or have previously been reported such as the antibody specific for sAPPalpha (clone 2D8).
7. Identify the source of cell lines and report if they were recently authenticated (e.g., by STR profiling) and tested for mycoplasma contamination.	HEK Flp-In 293 and THP-1 cell lines were purchased from Thermo Fisher Scientific and obtained from Roche (Basel, Switzerland), respectively. Cell lines were regularly tested for mycoplasma contamination and always found to be negative.

* for all hyperlinks, please see the table at the top right of the document

D- Animal Models

8. Report species, strain, gender, age of animals and genetic modification status where applicable. Please detail housing and husbandry conditions and the source of animals.	NA
9. For experiments involving live vertebrates, include a statement of compliance with ethical regulations and identify the committee(s) approving the experiments.	NA
10. We recommend consulting the ARRIVE guidelines (see link list at top right) (PLoS Biol. 8(6), e1000412, 2010) to ensure that other relevant aspects of animal studies are adequately reported. See author guidelines, under 'Reporting Guidelines'. See also: NIH (see link list at top right) and MRC (see link list at top right) recommendations. Please confirm compliance.	NA

E- Human Subjects

11. Identify the committee(s) approving the study protocol.	NA
12. Include a statement confirming that informed consent was obtained from all subjects and that the experiments conformed to the principles set out in the WMA Declaration of Helsinki and the Department of Health and Human Services Belmont Report.	NA
13. For publication of patient photos, include a statement confirming that consent to publish was obtained.	NA
14. Report any restrictions on the availability (and/or on the use) of human data or samples.	NA
15. Report the clinical trial registration number (at ClinicalTrials.gov or equivalent), where applicable.	NA
16. For phase II and III randomized controlled trials, please refer to the CONSORT flow diagram (see link list at top right) and submit the CONSORT checklist (see link list at top right) with your submission. See author guidelines, under 'Reporting Guidelines'. Please confirm you have submitted this list.	NA
17. For tumor marker prognostic studies, we recommend that you follow the REMARK reporting guidelines (see link list at top right). See author guidelines, under 'Reporting Guidelines'. Please confirm you have followed these guidelines.	NA

F- Data Accessibility

18. Provide accession codes for deposited data. See author guidelines, under 'Data Deposition'. Data deposition in a public repository is mandatory for: a. Protein, DNA and RNA sequences b. Macromolecular structures c. Crystallographic data for small molecules d. Functional genomics data e. Proteomics and molecular interactions	NA
19. Deposition is strongly recommended for any datasets that are central and integral to the study; please consider the journal's data policy. If no structured public repository exists for a given data type, we encourage the provision of datasets in the manuscript as a Supplementary Document (see author guidelines under 'Expanded View' or in unstructured repositories such as Dryad (see link list at top right) or Figshare (see link list at top right).	NA
20. Access to human clinical and genomic datasets should be provided with as few restrictions as possible while respecting ethical obligations to the patients and relevant medical and legal issues. If practically possible and compatible with the individual consent agreement used in the study, such data should be deposited in one of the major public access-controlled repositories such as dbGAP (see link list at top right) or EGA (see link list at top right).	NA
21. As far as possible, primary and referenced data should be formally cited in a Data Availability section. Please state whether you have included this section. Examples: **Primary Data** Wetmore KM, Deutschbauer AM, Price MN, Arkin AP (2012). Comparison of gene expression and mutant fitness in Shewanella oneidensis MR-1. Gene Expression Omnibus GSE39462 **Referenced Data** Huang J, Brown AF, Lei M (2012). Crystal structure of the TRBD domain of TERT and the CR4/5 of TR. Protein Data Bank 4O26 AP-MS analysis of human histone deacetylase interactions in CEM-T cells (2013). PRIDE PXD000208	NA
22. Computational models that are central and integral to a study should be shared without restrictions and provided in a machine-readable form. The relevant accession numbers or links should be provided. When possible, standardized format (SBML, CellML) should be used instead of scripts (e.g. MATLAB). Authors are strongly encouraged to follow the MIRIAM guidelines (see link list at top right) and deposit their model in a public database such as Biomodels (see link list at top right) or JWS Online (see link list at top right). If computer source code is provided with the paper, it should be deposited in a public repository or included in supplementary information.	NA

G- Dual use research of concern

23. Could your study fall under dual use research restrictions? Please check biosecurity documents (see link list at top right) and list of select agents and toxins (APHIS/CDC) (see link list at top right). According to our biosecurity guidelines, provide a statement only if it could.	NA