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Loss of tubulin deglutamylase CCP1 causes infantile-onset neurodegeneration

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(Note: Please note that the manuscript was previously reviewed at another journal and the reports were taken into account in the decision making process at The EMBO Journal. Since the original reviews are not subject to EMBO's transparent review process policy, the reports and author response cannot be published here. 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 12th Sep 2018

Thank you for submitting your manuscript to The EMBO Journal. The paper and the related one (MS 100440) have now been reviewed by one arbitrating advisor (referee #1) one who also had access to the previous referee comments from the other journal and your point-by-point response. This paper was also seen by an additional clinical expert (referee #2)

As you can see below both referees are very supportive of publication here. There are just a few text changes needed. Referee #2 is also asking if you have done any genotype/phenotype correlations. If so would be good to include that in the revised version.

I am therefore very happy to invite you to submit a revised version.

Congratulations on a nice study!

REFEREE REPORTS:

Referee #1:

The paper by Magiera et al uses mouse genetics to demonstrate the causal connection between increased microtubule polyglutamylation and neurodegeneration. By elegantly combining conditional knockouts of the deglutamylase CCP1 and polyglutamylase TTLL1, the authors show that excessive microtubule polyglutamylation induces degeneration of cerebellar Purkinje cells in a cell-autonomous manner. The authors then use double CCP1/CCP6 knockout mice to demonstrate that excessive polyglutamylation is toxic to other neuronal populations, thus proving that the observed phenotype does not represent a peculiarity of Purkinje cells. Importantly, the authors also convincingly show that this phenotype cannot be explained by enhanced microtubule severing by spastin, which is currently the best known candidate for a microtubule regulator responsive to polyglutamylation levels. Finally, the authors demonstrate that excessive polyglutamylation perturbs transport of mitochondria in cultured neurons.

An accompanying manuscript by Shashi et al. demonstrates the medical relevance of these observations by identifying recessive mutations in the CCP1-encoding gene as the cause of childhood-onset neurodegeneration in humans. The authors also demonstrate degeneration of peripheral nerve and spinal cord neurons in the CCP1-deficient mouse model, which fits nicely with the observations in patients.

The two manuscripts are complementary, and together, they represent a very important addition to the field, because they provide a convincing demonstration that abnormalities in microtubule post-translational modifications can lead to neurodegenerative disease. Both papers can be published after minor textual adjustments.

1. Magiera et al: the authors use hippocampal neurons as an established neuronal culture model for their transport studies. However, it is not clear whether hippocampal defects, such as neurodegeneration in this brain region, are present in CCP1/CCP6 knockout mice. The authors should comment on this in the main text of the paper. Furthermore, the authors use young (DIV4) neurons in their experiments. However, such young neurons may represent a better model for neurodevelopment rather than neurodegeneration. Again, the authors should comment on this in the main text of the paper.
Another point that requires some clarification is the efficiency of Cre-mediated deletion of CCP1 and CCP6-encoding genes in cultured neurons. It is clear that the levels of polyglutamylated tubulin are increased, but are the authors sure that both alleles of each deglutamylase are deleted? The authors should at least comment on this in the main text of the manuscript.
In the Discussion, the authors could give at least some attention to the potential origin of the observed transport defect and the lack of rescue of CCP1 deficiency in the spastin knockout mouse.

2. Shashi et al.: The explanation of the experiments shown in Fig. 3E and F is confusing, because the two completely different experiments, one of which in fact covers only one mutant, are described in the same short sentence on p.8. A much better description is required here. The authors should also present in the main or Expanded View figures the data listed as "not shown" or remove the corresponding statement, because the EMBO J does not permit citation of "Data not shown".

Referee #2:

This manuscript reports the identification of mutations in the CCP1 gene in humans with an early onset neurodevelopmental/neurodegenerative disease characterized by sever cerebellar atrophy. They also study a well established mouse model of CCP1 deficiency extending the mouse phenotype that now includes key features found in the human disease.
This is a well written manuscript that proves beyond any reasonable doubt that mutations in CCP1 causes a human neurological disease, they define the human phenotype, which is broad, by studying 13 patients. They establish that the mouse model is a very good, though not perfect, animal model for this human disease.
It would have been nice if they would have looked for genotype/phenotype correlations. There seem to be 3 "forms" of the disease - sever, relatively mild and intermediate.

1st Revision - authors' response 10th Oct 2018

We wish to thank both referees for their positive comments on our work.

Ad Referee #1:

Referee's comment: *The explanation of the experiments shown in Fig. 3E and F is confusing, because the two completely different experiments, one of which in fact covers only one mutant, are described in the same short sentence on p.8. A much better description is required here.*
Authors' reply: We have modified the wording changed the order in which the data are presented in the text (page 8, lines 3-7) and in the respective figure (see new version of Figure 3 and revised figure legend (page 29, lines 12-15).

Referee's comment: *The authors should also present in the main or Expanded View figures the data listed as "not shown" or remove the corresponding statement, because the EMBO J does not permit citation of "Data not shown".*
Authors' reply: We have changed the wording of the text in the Results section (page 9, lines 6-9) and present quantification of myelinated axons in a pure sensory nerve in new Appendix Figure S4.

Ad Referee #2:

Referee's comment: *It would have been nice if they would have looked for genotype/phenotype correlations. There seem to be 3 "forms" of the disease - severe, relatively mild and intermediate.*
Authors' reply: We thank the referee for raising this point. Although the number of 13 patients is probably too small to make definite statements and although there is no explanation at the molecular level, there might indeed be a genotype-phenotype correlation in terms of disease severity. We have added a few sentences in the Results (page 7, lines 16-18) and Discussion (page 10, lines 7-12) sections.

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PLEASE NOTE THAT THIS CHECKLIST WILL BE PUBLISHED ALONGSIDE YOUR PAPER

Corresponding Author Name: Dr. Jan Senderek
Journal Submitted to: The EMBO Journal
Manuscript Number: EMBOJ-2018-100540

Reporting Checklist For Life Sciences Articles (Rev. June 2017)

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.

In the pink boxes below, please ensure that the answers to the following questions are reported in the manuscript itself. Every question should be answered. If the question is not relevant to your research, please write NA (non applicable). We encourage you to include a specific subsection in the methods section for statistics, reagents, animal models and human subjects.

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?	Whenever results from molecular biological studies were quantified, sample size was chosen to be at least 3 experiments per condition to be able to make statistical analyses.
1.b. For animal studies, include a statement about sample size estimate even if no statistical methods were used.	For mouse studies, a minimum of 3 animals per condition were analyzed.
2. Describe inclusion/exclusion criteria if samples or animals were excluded from the analysis. Were the criteria pre-established?	For mouse studies, all animals within a group were analyzed.
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 specific randomization procedures were used.
For animal studies, include a statement about randomization even if no randomization was used.	No specific randomization procedures were used.
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.	For histology, histochemistry, and electron microscopy, mouse tissues were analysed as numbered samples. The person analysing the samples was not aware of the genotypes.
4.b. For animal studies, include a statement about blinding even if no blinding was done	For histology, histochemistry, and electron microscopy, mouse tissues were analysed as numbered samples. The person analysing the samples was not aware of the genotypes.
5. For every figure, are statistical tests justified as appropriate?	Details of statistical tests used are mentioned in the figure legends.
Do the data meet the assumptions of the tests (e.g., normal distribution)? Describe any methods used to assess it.	Normal distribution of data was assessed by visual inspection of the distribution.
Is there an estimate of variation within each group of data?	Standard deviations are provided as measures of variation.
Is the variance similar between the groups that are being statistically compared?	The variance was similar between the groups that were statistically compared.

C- Reagents

USEFUL LINKS FOR COMPLETING THIS FORM

http://www.antibodypedia.com
http://1degreebio.org
http://www.equator-network.org/reporting-guidelines/improving-bioscience-research-repo

http://grants.nih.gov/grants/olaw/olaw.htm
http://www.mrc.ac.uk/Ourresearch/Ethicsresearchguidance/Useofanimals/index.htm
http://ClinicalTrials.gov
http://www.consort-statement.org
http://www.consort-statement.org/checklists/view/32-consort/66-title

http://www.equator-network.org/reporting-guidelines/reporting-recommendations-for-tun

http://datadryad.org

http://figshare.com

http://www.ncbi.nlm.nih.gov/gap

http://www.ebi.ac.uk/ega

http://biomodels.net/

http://biomodels.net/miriam/
http://jjj.biochem.sun.ac.za
http://oba.od.nih.gov/biosecurity/biosecurity_documents.html
http://www.selectagents.gov/

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).	Details are provided in the Materials & Methods section.
7. Identify the source of cell lines and report if they were recently authenticated (e.g., by STR profiling) and tested for mycoplasma contamination.	HEK293 cells were obtained from ATCC. Cells tested negattive for mycoplasma contamination. No further authentication was performed (this study used these cells as "bioreactors" to overexpress CCP1 but did not use these cells for disease modelling).

* 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.	pcd (pcd3J) mice (BALB/cByJ-Agtpbp1pcd-3J/J; www.jax.org/strain/003237) were obtained from The Jackson Laboratory and backcrossed on the C57BL/6N background.
9. For experiments involving live vertebrates, include a statement of compliance with ethical regulations and identify the committee(s) approving the experiments.	Animal care and use for this study were performed in accordance with the recommendations of the European Community (2010/63/UE) for the care and use of laboratory animals. Experimental procedures were specifically approved by the ethics committee of the Institut Curie in compliance with the international guidelines.
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.	Our animal studies are in line with all major animal study guidelines. Our study was approved by an ethics committee.

E- Human Subjects

11. Identify the committee(s) approving the study protocol.	Page 12, 1st paragraph
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.	Page 12, 1st paragraph
13. For publication of patient photos, include a statement confirming that consent to publish was obtained.	No patient photographs are published in this study.
14. Report any restrictions on the availability (and/or on the use) of human data or samples.	Availability of further human (genomic and clinical) data and human samples is restricted (according to the consent agreements used). The individual investigative teams can ask patients and families for re-consent on an individual basis, if requested.
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 a "Data Availability" section at the end of the Materials & Methods, listing the accession codes for data generated in this study and deposited in a public database (e.g. RNA-Seq data: Gene Expression Omnibus GSE39462, Proteomics data: PRIDE PXD000208 etc.) Please refer to our author guidelines for '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	There is no such data generated in our study.
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).	There is no such data generated in our study.
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).	Sharing of complete genomic ("exome") datasets and extended clinical details of patients involved in this study would not be compatible with the consent agreements used. If requested, patients can be re-consented on an individual basis for further sharing of data.
21. 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.	There is no such data generated in our study.

G- Dual use research of concern

22. 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