EMBO
reports

Manuscript EMBOR-2012-36857

Charcot-Marie-Tooth disease associated mutants of GDAP1 dissociate its roles in peroxisomal and mitochondrial fission

Nina Huber, Sofia Guimaraes, Michael Schrader, Ueli Suter and Axel Niemann

Corresponding author: Axel Niemann, Institute of Molecular Health Sciences, Cell Biology

Review timeline:

Submission date:	15 November 2012
Editorial Decision:	20 December 2012
Revision received:	13 March 2013
Correspondence:	08 April 2013
Accepted:	11 April 2013

Editor: Barbara Pauly

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 20 December 2012

Thank you very much for the submission of your research manuscript to our editorial office. Please accept my apologies for the unusual delay in the review process of your study, which was due to the upcoming holiday season in which referees usually take more time to submit their reports. We have just now received the full set of reviews on your manuscript.

You will see that all reviewers appreciate the interest of your findings and support publication of your study in our journal. Referees 1 and 2 only have minor concerns that can be addressed in writing. Referee 3 also brings up some points that would need to be addressed experimentally, but it seems as if these are rather minor things.

Given these positive evaluations, the reviewers constructive comments and the potential interest of the study, I would like to give you the opportunity to revise your manuscript, with the understanding that the concerns of the reviewers should be addressed. Acceptance of the manuscript will depend on a positive outcome of a second round of review and I should also remind you that it is EMBO reports policy to allow a single round of revision only and that therefore, acceptance or rejection of the manuscript will depend on the completeness of your responses included in the next, final version of the manuscript.

Revised manuscripts should be submitted within three months of a request for revision; they will otherwise be treated as new submissions. If you feel that this period is insufficient for a successful submission of your revised manuscript I can potentially extend this period slightly. Also, the length of the revised manuscript should not exceed roughly 29,000 characters (including spaces). It is currently slightly longer than this, and especially if additional figures/text is included in the revision, some shortening might be required. You may consider including some peripheral data in the form of Supplementary information. However, materials and methods essential for the repetition of the key experiments should be described in the main body of the text and may not be displayed as supplemental information only.

We have also started encouraging authors to submit the raw data of biochemical and/or microscopical images to our editorial office. These data will be published online as part of the supplementary information. This is voluntary at the moment, but if you agree that this would be useful for readers I would like to invite you to supply these files when submitting the revised version of your study.

As part of the EMBO publication's Transparent Editorial Process, EMBO reports publishes online a Review Process File to accompany accepted manuscripts. This File will be published in conjunction with your paper and will include the referee reports, your point-by-point response and all pertinent correspondence relating to the manuscript.

You are able to opt out of this by letting the editorial office know (emboreports@embo.org). If you do opt out, the Review Process File link will point to the following statement: "No Review Process File is available with this article, as the authors have chosen not to make the review process public in this case."

We also welcome the submission of cover suggestions or motifs that might be used by our Graphics Illustrator in designing a cover.

I look forward to seeing a revised form of your manuscript when it is ready. Should you in the meantime have any questions, please do not hesitate to contact me.

REFEREE REPORTS:

Referee #1:

Huber and colleagues have identified and characterized a role for the ganglioside induced associated protein-1 (GDAP1) in peroxisome as well as mitochondrial function. They demonstrate that most missense mutations that cause Charcot Marie Tooth disease do not alter the peroxisomal function of GDAP1 though they do affect fission introduced in mitochondria. Therefore there are differences in how GDAP1 regulates peroxisome fragmentation compared to mitochondrial fragmentation despite the fact that, as the authors convincingly show, the C-terminal TA and HD1 domains are required for both organelles. Moreover both organelles require interactions with Drp1 and Mff. It is interesting that the one recessive CMT mutation that truncates the TA domain and does alter peroxisomal fission causes a more severe peripheral neuropathy in patients. Additional comments on the manuscript are as follows:

1) The paper is well written and the figures are clear and support the data.
2) The results advance this field. Much of what is known about the interesting GDAP1 protein comes from this laboratory. For example it is because of these investigators that we know that GDAP1 acts on mitochondrial fission and that disease causing mutations disrupt mitochondrial fission. The current work extends knowledge by clearly demonstrating a role for GDAP1 in regulating peroxisomal fission. The fact that this may also correlate with disease severity in this common recessive form of CMT is an added bonus to this work.
3) The results extend beyond the CMT field as understanding the molecular basis fusion and fission in organelles like mitochondria and peroxisomes provides basic knowledge of the cellular biology of these processes.
4) A minor request would be to link the morphological abnormalities more clearly to the biology.

For example the authors clearly state that the steps in fission begin with spherical peroxisomes that then elongate before undergoing fragmentation/division and returning to a spherical state. When various constructs cause them to remain in an elongated or tubular state does this mean that they are arrested at this point along the path to fission or is there another explanation. Perhaps this point may seem obvious but without understanding this issue it makes it harder to interpret the biological context of the findings. This could be simply addressed in a sentence or two.

Referee #2:

This is a well written study by competent investigators.

The paper gives new light on the possible mechanism of disease in CMT with GDAP1 mutations. However how these changes might produce both dominant or recessive disease is worthy of more detailed discussion.

The paper gets complicated at the end where different mechanisms of different mutations are discussed. Some clarification is needed for the reader. Where these mechanisms might differ with different mutations should be outlined. This could be achieved in a summary schematic or table. It is clear that the authors do not wish to over intrerpret their data but this information could be presented as putative, as such a summary should lead to future studies. This should be done to add meaning to the studies, as such mechanisms may explain why some mutations are recessive and others dominant, this has always been a curious feature of GDAP1 CMT.

Referee #3:

In this manuscript authors investigate the role of GDAP1 in peroxisomal fission. Using a combination of approaches, they show that GDAP1 is targeted to peroxisomes in a Pex19-dependent manner and that levels of GDAP1 correlate with peroxisomal fragmentation. Interestingly, pathogenic mutants of GDAP1 are as efficient as wt GDAP1 in peroxisomal fission, highlighting a primary role for mitochondria in the pathogenesis of CMT.

In general this is a potentially interesting study that could elucidate the role of peroxisomes in the pathogenesis of CMT due to GDAP1 mutations. The experiments are quite convincing and the paper is going to be a nice addition to the field of mitochondrial and peroxisomal fission by the group who has led the research on GDAP1, but at this point there are some points to be addressed and clarified.

I have the following concerns that must be addressed
1. The title does not really reflect the main message of the manuscript: something like "disease associated mutants of GDAP1 dissociate its roles in mitochondrial and peroxisomal fission" would be more appropriate.
2. Fig1. This figure is crucial to assign localization to peroxisomes. I have several concerns
a. Improve quality and resolution of the stainings. In particular, it is difficult to appreciate in the pixelated images presented here that GDAP1 localizes also to peroxisomes. Increase the resolution by increasing the size of the scan in the confocal imaging setup used here (also, please provide some more experimental details)
b. Perform a triple staining for GDAP1, mitochondria and peroxisomes to show in the same cell how much of GDAP1 goes on each organelle
c. Add an experiment of subcellular fractionation to corroborate the finding of peroxisomal targeting
d. Show images supporting the quantitative data of Fig. 1C.
e. Correct the statistical analysis: SD is not appropriate for independent experiments, see the commentary by Vaux, JCB. Authors shall use SEM or CI if the experiments are independent as they state. Indicate which t test they are using (paired, two sample?)
3. I have difficulties in reconciling the results of the experiments of fig. 3e-f with the conclusion that the mutants used here dissociate peroxisomal and mitochondrial fission by GDAP1. It seems to me that the deletion mutants unable to fragment mitochondria are similarly unable to fragment peroxisomes. The authors shall extend this mutational analysis in the setting of the first two panels of this figure (i.e., after downregulation of endogenous GDAP1) to make the results comparable.

1st Revision - authors' response 13 March 2013

Answers to the referees' comments:

Referee #1:

A minor request would be to link the morphological abnormalities more clearly to the biology. For example the authors clearly state that the steps in fission begin with spherical peroxisomes that then elongate before undergoing fragmentation/division and returning to a spherical state. When various constructs cause them to remain in an elongated or tubular state does this mean that they are arrested at this point along the path to fission or is there another explanation. Perhaps this point may seem obvious but without understanding this issue it makes it harder to interpret the biological context of the findings. This could be simply addressed in a sentence or two.

We agree that this is an important point. To clarify this, we added a description on page 6 and at the beginning of the concluding remarks. In addition we adapted the wording throughout the manuscript to clearly describe the sequence of morphological changes.

Referee #2:

The paper gives new light on the possible mechanism of disease in CMT with GDAP1 mutations. However how these changes might produce both dominant or recessive disease is worthy of more detailed discussion.
The paper gets complicated at the end where different mechanisms of different mutations are discussed. Some clarification is needed for the reader. Where these mechanisms might differ with different mutations should be outlined. This could be achieved in a summary schematic or table. It is clear that the authors do not wish to over intrerpret their data but this information could be presented as putative, as such a summary should lead to future studies. This should be done to add meaning to the studies, as such mechanisms may explain why some mutations are recessive and others dominant, this has always been a curious feature of GDAP1 CMT.

As proposed by the reviewer we added a table to summarize our findings on peroxisomal fission. We also included the results of previous publications using the same experimental approaches and constructs to analyze the effects on mitochondrial fission and mitochondrial fusion (Supplementary Table 1).

In addition the reviewer suggests discussing the different mechanisms caused by dominantly and recessively inherited mutations. In the "Concluding Remarks" section we suggested that the N-terminal cytosolic domains of GDAP1 might have a putative different regulatory function in mitochondrial and peroxisomal fission. We also mention that dominantly and recessively inherited point mutations lie within these N-terminal domains. We do agree that this is an interesting point in CMT caused by mutations in *GDAP1*. However, we feel that additional speculative considerations on the different mutants will not significantly improve the discussion of our results while staying in the length constrains.

Referee #3:

1. The title does not really reflect the main message of the manuscript: something like "disease associated mutants of GDAP1 dissociate its roles in mitochondrial and peroxisomal fission" would be more appropriate.

We agree and have changed the title accordingly. Thank you!

2. Fig1. This figure is crucial to assign localization to peroxisomes. I have several concerns
a. Improve quality and resolution of the stainings. In particular, it is difficult to appreciate in

the pixelated images presented here that GDAP1 localizes also to peroxisomes. Increase the resolution by increasing the size of the scan in the confocal imaging setup used here (also, please provide some more experimental details)

We have repeated the stainings on primary hippocampal neurons to increase the resolution. As we changed the culture media conditions, we also updated the experimental procedures accordingly.

b. Perform a triple staining for GDAP1, mitochondria and peroxisomes to show in the same cell how much of GDAP1 goes on each organelle

Following the reviewer's suggestion we added a triple overlay of the blow-ups.

c. Add an experiment of subcellular fractionation to corroborate the finding of peroxisomal targeting.

Generally liver is used to purify peroxisomes. As GDAP1 is not expressed in mouse liver, we could use CNS tissue. The purification of peroxisomes from CNS tissue is possible, however, a clear separation of the light mitochondrial fraction and peroxisomes is very difficult to achieve. We discussed this issue with experts and authors of recent relevant papers and came to the conclusion that a cell fractionation will not add convincing evidence for the peroxisomal localization of GDAP1 (Markus Islinger (University of Heidelberg) and Werner Kovacs (ETH Zurich), personal communication). We feel that we present different lines of evidence to demonstrate convincingly that GDAP1 is targeted to peroxisomes, independent of the cell fractionation approach.

d. Show images supporting the quantitative data of Fig. 1C.

We have added a new Supplementary Figure 1 B to illustrate the quantifications.

The illustration is supportive to follow the quantification procedure. However, the quantifications in Fig 1C are based on at least 1000 peroxisomal GDAP1-intensities per condition in three independent experiments. Thus, we chose not to give these selected high magnification images the same emphasis, and added the illustrative images as supplementary data.

e. Correct the statistical analysis: SD is not appropriate for independent experiments, see the commentary by Vaux, JCB. Authors shall use SEM or CI if the experiments are independent as they state. Indicate which t test they are using (paired, two sample?)

We changed all error bars to standard error and clarified the test used.

3*. I have difficulties in reconciling the results of the experiments of fig. 3e-f with the conclusion that the mutants used here dissociate peroxisomal and mitochondrial fission by GDAP1. It seems to me that the deletion mutants unable to fragment mitochondria are similarly unable to fragment peroxisomes. The authors shall extend this mutational analysis in the setting of the first two panels of this figure (i.e., after downregulation of endogenous GDAP1) to make the results comparable.*

This question is partly overlapping with the comment of Reviewer #2. The new Supplementary Table 1 summarizes the effect of the different mutant forms of GDAP1 and is helpful for the interpretation of the presented results.
In addition, we agree with the reviewer's comment that the different experimental approaches used in this section might lead to confusion. Thus, we performed knockdown and rescue experiments with the deletion mutants, and we now present the results for the all mutations in the same experimental setup (Fig. 3A), as suggested. The new panels replace the panels E to F in Figure 3. The former Fig. 3 E-F is now combined in Supplementary Figure 5. Thus, the overexpression approach for disease-related mutants and for deletion mutants are now also shown together in one Figure.

© European Molecular Biology Organization

Correspondence - Editor 08 April 2013

Many thanks for the submission of your revised manuscript to our editorial office. Please accept my apologies for the delay in getting back to you with a decision on it. Your study was sent back to one of the original referees and I am happy to tell you that this referee now supports publication of your study in EMBO reports.

I think I brought to your attention the fact that we have started encouraging authors to submit the raw data of biochemical and/or microscopical images to our editorial office. These data will be published online as part of the supplementary information. This is voluntary at the moment, but if you agree that this would be useful for readers I would like to invite you to supply these files so that they can be published alongside the manuscript.

If you are willing to provide this data, you can simply send the files to us by email and I will upload them to your manuscript before accepting it officially.

2nd Editorial Decision 11 April 2013

Many thanks for submitting the source data of your figures to our editorial office. We have uploaded them to your manuscript and will publish them alongside with the article.

As part of the EMBO publication's Transparent Editorial Process, EMBO reports publishes online a Review Process File to accompany accepted manuscripts. As you are aware, this File will be published in conjunction with your paper and will include the referee reports, your point-by-point response and all pertinent correspondence relating to the manuscript.

If you do NOT want this File to be published, please inform the editorial office within 2 days, if you have not done so already, otherwise the File will be published by default [contact: emboreports@embo.org]. If you do opt out, the Review Process File link will point to the following statement: "No Review Process File is available with this article, as the authors have chosen not to make the review process public in this case."

Finally, we provide a short summary of published papers on our website to emphasize the major findings in the paper and their implications/applications for the non-specialist reader. To help us prepare this short, non-specialist text, we would be grateful if you could provide a simple 1-2 sentence summary of your article in reply to this email.

Thank you again for your contribution to EMBO reports and congratulations on a successful publication. Please consider us again in the future for your most exciting work.

© European Molecular Biology Organization