

Manuscript EMBO-2009-73479

## Suppression of the novel ER protein MAXER by mutant ataxin-1 in Bergman glia contributes to non-cell autonomous toxicity

Hiroki Shiwaku, Natsue Yoshimura, Takuya Tamura, Masaki Sone, Soichi Ogishima, Kei Watase, Kazuhiko Tagawa, Hitoshi Okazawa

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### Review timeline:

|                     |                  |
|---------------------|------------------|
| Submission date:    | 21 December 2009 |
| Editorial Decision: | 26 January 2010  |
| Revision received:  | 08 April 2010    |
| Editorial Decision: | 10 May 2010      |
| Revision received:  | 12 May 2010      |
| Accepted:           | 14 May 2010      |

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### 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

26 January 2010

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Thank you for submitting your manuscript for consideration by The EMBO Journal, and please excuse the somewhat delayed evaluation at this time around the turn of the years. We have now received the reports of three expert reviewers, whose comments directly to the authors are copied below. As you will see, all three reviewers consider your findings potentially interesting, and commend on the comprehensiveness of the analysis. Nevertheless, they also raise a significant number of concerns and issues that preclude publication in the present form. Although I realize that carefully addressing all these various points may require a substantial amount of additional time and effort, I would given the importance of the subject and the overall interest of your results still be inclined to allow you the opportunity to address the referees' comments through a revised version of the manuscript. For such a revision to be successful, it will however be important to provide better descriptions of experimental procedures and details, as well as the requested experimental controls and improvements of other technical points. Likewise, it would certainly be helpful to alter the presentation as suggested by the reviewers, including language editing to better convey the main messages of the study, improving the discussions and interpretations of the findings, and streamlining the results and data panels according to their importance in supporting the main conclusions of the work. At the same time, all referees also request additional experimental data to extend the understanding of various aspects of the presented work - while I appreciate that addressing all of these points may go beyond the scope of this already rather comprehensive study, I realize that some of these suggestions may indeed be helpful to round up the conclusions on the role of MAXER in Bergmann glia and SCA1 pathogenesis; I would in this case be happy to discuss these points further if necessary.

In conclusion, should you be able to satisfactorily substantiate the study in the spirit of the referees' comments, we should be happy to consider a revised manuscript for publication. As it is EMBO Journal policy to allow a single round of major revision only, it will however be important to diligently answer to all the various points raised at this stage if you wish the manuscript ultimately to be accepted. When preparing your letter of response, please also bear in mind that this will form part of the Review Process File, and will therefore be available online to the community in the case of publication (for more details on our Transparent Editorial Process initiative, please visit our website: <http://www.nature.com/emboj/about/process.html>). In any case, as indicated above please do not hesitate to get back to us should you need feedback on any issue regarding your revision.

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

Yours sincerely,

Editor  
The EMBO Journal

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#### REFEREE REPORTS:

##### Referee #1 (Remarks to the Author):

This manuscript by Shiwaku et al., entitled "MAXER, a novel ER membrane protein, possibly mediates non-cell autonomous toxicity of mutant ataxin-1 via Bergmann glia," describes an extensive array of studies to determine the role of a novel gene, MAXER, in the pathogenesis of SCA1. The authors show data that reveal MAXER to be an ER-microtubule-linking protein, which is downregulated by expanded ataxin-1 expression. The authors show data that this downregulation of MAXER leads to decreased Cyclin D1 levels and accumulation of cells in G1. The idea is that a lack of proliferating Bergmann glia, due to decreased MAXER in response to expanded ataxin-1, leads to the early window of vulnerability in SCA1. While the premise is extremely interesting, the data supporting some of the conclusions is incomplete. The following points need to be addressed.

1. One main issue is the question of the role of MAXER in the decreased GLAST expression seen in the presence of expanded Ataxin-1 expression. The authors show in Figure 6 B, C that GLAST signal intensity parallels Bergmann glial cell number, suggesting that the loss of GLAST is secondary to the loss of Bergmann glial cells. However, in Figure 7, the authors show that expanded Ataxin-1 expression leads to a decrease in GLAST expression in surviving Bergmann glia; GLAST expression is rescued by MAXER overexpression in these cells. How decreased MAXER expression is involved in decreased GLAST expression is unclear, and the authors do not adequately discuss this point. Moreover, the similar decrease in GLAST expression and Bergmann glial cell number shown in Figure 6 are inconsistent with the idea that expanded Ataxin-1 decreases GLAST expression directly or indirectly.
2. The statement that nocodazole disrupts the interaction between MAXER and microtubules is not well substantiated by the immunostaining (Fig. 3). Biochemical verification of the loss of MAXER/alpha-tubulin interaction is needed under nocodazole conditions. It appears that MAXER co-IPs with monomeric alpha-tubulin, and thus, the disruption by nocodazole might not really be a loss of MAXER binding to alpha-tubulin but simply the disruption of the microtubule structure by nocodazole.
3. A nuclear localization signal is illustrated in the diagrams of MAXER domain structure. This domain and its role in MAXER function should be discussed.
4. Figure 5E shows only one cell with Maxer shRNA expression; this is insufficient to make this point, even with the quantification shown in Fig. 5F.
5. To support the authors' hypothesis, it would be nice to see that forced expression of CDK5RAP3 rescues Cyclin D1 expression.
6. The transfection and infection efficiencies are not described for any experiment, but should be for all. Examples include, but are not limited to, Fig. 5I. This is critical as well for Fig. 7.
7. One of the most interesting and important experiments is shown in Fig. 7. However, it is hard to

know, in the Bergmann glial cell experiments shown in Figure 7A, how much of the decrease in Bergmann glial cell number is due to decreased proliferation vs. cell death. Is expanded Ataxin-1 simply inhibiting Bergmann glial cell division or is it also directly inducing death by a (presumably) MAXER-independent pathway? Some analysis of this is surely needed.

8. The MAXER knockdown studies in Supp. Fig. 6 suggest that MAXER plays a role in Bergmann glia proliferation and function. This limited analysis does not indicate that MAXER is the downstream effector of mutant Atx1 in SCA1. Analysis of the knockout mouse would be needed for that conclusion.

9. The paper suffers from relatively little detail given to describe many of the experiments.

10. Better protein loading in Fig. 1C is needed to compare MAXER levels between tissues.

11. This is an enormous paper with many figures, including supplemental. Many of the supplemental figures should be within the body of the paper, rather than supplemental. More judicious use of the figures would enhance the manuscript. For example, is the experiment shown in Supp. Fig. 9 really central to the paper?

12. The description of the binding domain of MAXER with alpha-tubulin (Fig. 3E) should be better described within the text (p. 7). In addition, the description of the specific MAXER domain responsible for binding CDK5RAP3 is also needed in the text.

Referee #2 (Remarks to the Author):

Via microarray assays, the authors found that Maxer, a novel protein, is downregulated in cultured SCA1 cells. They further found that Maxer is an ER associated protein and is expressed in Bergmann glia. Loss of Maxer inhibits the proliferation of glial cells as well as glutamate transporter GLAST, while overexpression of Maxer can rescue them.

The studies were well done. Many approaches and experiments were nicely coordinated to generate interesting results. The normal function of Maxer appears to be characterized. However, there are two general issues about the role of Maxer in the pathogenesis of SCA1.

The first one is that, as Maxer is also expressed in neuronal cells such as Purkinje cells, it is unclear whether reduction of Maxer in neurons is sufficient to induce neurotoxicity or neurodegeneration. Knowing this would help evaluate the role of glial Maxer in SCA1. Also, more quantitative results about the loss of Bergmann glial cells and Maxer in SCA1 mouse brains need to be provided, such as western blotting that is more quantitative than fluorescent imaging.

The other issue is why mutant ataxin-1 decreases the transcription of Maxer.

I think that if the authors could provide further insight into the above issues, the manuscript would be much more interesting.

Specific issues

1. The reduction of Maxer was found in cultured cells transfected or infected with mutant Ataxin-1. The authors need to provide clear evidence that Maxer is reduced in Ataxin-1 knock-in mouse brains via western blotting of several mouse brain samples. Glial marker protein should also be probed on the blots to verify that the equal amounts of glial cells were examined.

2. Some important results were from cultured cells that were transfected with Maxer or its fusion protein with GFP. The paper would be strengthened if there were more *in vivo* evidence for the subcellular distribution of endogenous Maxer. For example, figure 3 needs to show the association of endogenous Maxer, but not transfected GFP-Maxer, with ER. GFP-Maxer's subcellular localization is not very convincing, as the image resolution is not high enough.

3. The authors need to provide evidence via western blotting to show if there is decrease of SOX2 in the cerebellum tissue, which can verify the decrease of Bergmann glia in ataxin-1.

4. The authors focus on GLAST for glutamate transporter expression. They should also examine GLT-1, which is also important for up-taking glutamate by glial cells.

Referee #3 (Remarks to the Author):

The manuscript by Shiwaku et al describes the identification of a novel ER protein that is downregulated by mutant ataxin-1 and leads to negatively impacts the development and function of cerebellar Bergmann glia. The authors propose the hypothesis that the effect of mutant ataxin-1 expression on Bergmann glia contributes to the pathogenesis of SCA1. A very large amount of data is presented in this manuscript, making it very difficult to evaluate. Nevertheless, there are a number of important experiments presented here that if distilled down to those critical for the author's main points could be come a communication that would be very useful to other investigators in the polyglutamine neurodegeneration field.

I would suggest a number of revisions to improve this manuscript.

- 1) The first and most critical point for the authors of this manuscript is to obtain assistance from an editor, experienced in writing in English, who can assist them in assuring their ideas are clearly communicated using well constructed scientific English.
- 2) A number of figures, sub figures and supplemental figures are not needed for the author's to make the main points of their paper and simply add confusion for a reader trying to glean the importance of this protein to the pathogenesis of SCA1. In addition, some figures make points the author's point unconvincingly. I would recommend removing Fig. 1b, Fig. 3, Fig. 4a and 4e while moving the rest of Fig. 4 to supplementary figures, reformatting Fig 5 into two figures and removing supplementary figs. 1, 2 and 4.
- 3) Figure 2D and 2E would be more convincing if the nuclear and cytosolic markers were switched (Maxer w/ Sox 2 and Ataxin 1 w/ GFAP) because it looks like the are imaging bleed-through fluorescence.
- 4) While I believe Fig. 3 should be removed because it is not really relevant to the main point of the paper, the images in it are also unconvincing. The images in 3D and 3F do not make the point well at all. Most of the images picked to show the ER pattern do so convincingly, but the images picked to show the co-localization w/ tubulin have a completely different pattern of localization. This suggests that there is a strong likelihood that one or the other images are impacted by bleed through fluorescence.
- 5) The FACS analysis showing % of cells in G1 in Fig.'s 4 and 5 are not convincing. Is this really significantly different? Information about positive and negative controls and number of experimental replicates would help.
- 6) The authors should discuss how it happened that they found this protein more clearly. The way it is presented it is not clear because it says they found it during a proteomic and genomic analysis of cerebellar neurons. Did they find it by proteomics, expression profiling or both? Which neurons were they studying? Cultured neurons? Were glia present in the cultures? If not, why do they think they found a glial protein in theses studies?
- 7) The authors need to discuss the issue that mutant ataxin-1 clearly has cell autonomous toxicities in Purkinje neurons as well, since transgenic mice expressing the mutant protein under a Purkinje neuron specific promoter develop ataxia and Purkinje neuron degeneration. While Maxer may play a role in preventing normal Bergmann glia function and thus make the cerebellum more vulnerable to injury, Maxer is not expressed in Purkinje neurons. Thus, it is still questionable whether the role of Maxer is a major contributor to SCA1 pathogenesis or simply a concurrent finding. The clinical course of the disease (neurodegenerative, not neurodevelopmental) suggests that the finding they are reporting of abnormal development and proliferation of Bergmann glia may not be of clinical importance. The data should be presented in this context.

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 Referee #1 (Remarks to the Author):

*This manuscript by Shiwaku et al., entitled "MAXER, a novel ER membrane protein, possibly mediates non-cell autonomous toxicity of mutant ataxin-1 via Bergmann glia," describes an extensive array of studies to determine the role of a novel gene, MAXER, in the pathogenesis of SCA1. The authors show data that reveal MAXER to be an ER-microtubule-linking protein, which is downregulated by expanded ataxin-1 expression. The authors show data that his downregulation of MAXER leads to decreased Cycin D1 levels and accumulation of cells in G1. The idea is that a lack of proliferating Bergmann glia, due to decreased MAXER in response to expanded ataxin-1, leads to the early window of vulnerability in SCA1. While the premise is extremely interesting, the data supporting some of the conclusions is incomplete. The following points need to be addressed.*

We thank the reviewer for this high evaluation of our manuscript. We corrected the manuscript as following.

*1. One main issue is the question of the role of MAXER in the decreased GLAST expression seen in the presence of expanded Ataxin-1 expression. The authors show in Figure 6 B, C that GLAST signal intensity parallels Bergmann glial cell number, suggesting that the loss of GLAST is secondary to the loss of Bergmann glial cells. However, in Figure 7, the authors show that expanded Ataxin-1 expression leads to a decrease in GLAST expression in surviving Bergmann glia; GLAST expression is rescued by MAXER overexpression in these cells. How decreased MAXER expression is involved in decreased GLAST expression is unclear, and the authors do not adequately discuss this point. Moreover, the similar decrease in GLAST expression and Bergmann glial cell number shown in Figure 6 are inconsistent with the idea that expanded Ataxin-1 decreases GLAST expression directly or indirectly.*

To answer the question from the reviewer, we repeated several experiments. We reexamined the signal intensities of GLAST, which make clearer the discrepancy between reduction of Bergmann glia number and GLAST signal intensity (Figure 6D). We also found in WB that reduction of GLAST is higher than reduction of Sox2 (Figure 6C and 6E). Figure 7C supports that GLAST signal/Bergmann glia is decreased, as the reviewer commented. Thus, both (Bergmann cell number) and (GLAST/ a Bergmann cell) contributed to GLAST reduction. We discussed about this point in the discussion of our revised manuscript (page 14, last paragraph).

*2. The statement that nocodazole disrupts the interaction between MAXER and microtubules is not well substantiated by the immunostaining (Fig. 3). Biochemical verification of the loss of MAXER/alpha-tubulin interaction is needed under nocodazole conditions. It appears that MAXER co-IPs with monomeric alpha-tubulin, and thus, the disruption by nocodazole might not really be a loss of MAXER binding to alpha-tubulin but simply the disruption of the microtubule structure by nocodazole.*

We checked the MAXER/alpha-tubulin interaction needed under nocodazole conditions, and added the data in Reviewer Only Figure. This was a little bit unexpected because the result suggested interaction between monomer tubulin and Maxer even when microtubules are disrupted. However, since reviewer 3 and the editor (we contacted him about this point) recommended us to delete Figure 3D, E, and F, we omitted this part.

*3. A nuclear localization signal is illustrated in the diagrams of MAXER domain structure. This domain and its role in MAXER function should be discussed.*

We added the data showing that deletion of NLS change the localization of Maxer (Supplementary figure 2). Meanwhile for the function of NLS, transmembrane domain is inhibitory because FL-Maxer sticks to ER by TM. We discussed about this point in the text (page 7).

4. *Figure 5E shows only one cell with Maxer shRNA expression; this is insufficient to make this point, even with the quantification shown in Fig. 5F.*

We added a new image to multiple cells expressing Maxer shRNA (new Figure 5B).

5. *To support the authors' hypothesis, it would be nice to see that forced expression of CDK5RAP3 rescues Cyclin D1 expression.*

This seems misunderstanding of the reviewer. CDK5RAP3 is an inhibitor of Cyclin D1, thus we added data that siRNA of CDK5RAP3 rescued Cyclin D1 (Figure 5F) in addition to previous data about its rescue for G1 (Figure 5H).

6. *The transfection and infection efficiencies are not described for any experiment, but should be for all. Examples include, but are not limited to, Fig. 5I. This is critical as well for Fig. 7.*

We newly added figures for the transfection and infection efficiencies (Supplementary figure 3), in which we show representative images of transfection and infection. On the image panels, the efficiencies are shown in percentage (Supplementary figure 3).

7. *One of the most interesting and important experiments is shown in Fig. 7. However, it is hard to know, in the Bergmann glial cell experiments shown in Figure 7A, how much of the decrease in Bergmann glial cell number is due to decreased proliferation vs. cell death. Is expanded Ataxin-1 simply inhibiting Bergmann glial cell division or is it also directly inducing death by a (presumably) MAXER-independent pathway? Some analysis of this is surely needed.*

To answer this critical question, we added analysis of cell death in vitro and in vivo (Supplementary figure 6). Our in vitro data corresponding to Figure 7A showed no death is induced in cerebellar cell cultures. Therefore, the decrease of Bergmann glia is due to decreased proliferation but no to death. Consistently, in vivo cerebellum at P7 showed no death in Purkinje cell layer where Bergmann glia exist.

8. *The MAXER knockdown studies in Supp. Fig. 6 suggest that MAXER plays a role in Bergmann glia proliferation and function. This limited analysis does not indicate that MAXER is the downstream effector of mutant Atx1 in SCA1. Analysis of the knockout mouse would be needed for that conclusion.*

We agree with the reviewer basically. Although we suspect that Maxer is a downstream effector because Maxer can rescue Atx1-induced decrease of Bergmann glia (Figure 7), it is true that the similar experiment at the level of knockout/Tg mouse will be necessary to conclude. We asked the editor about this point, and he agreed that it is beyond the scope of this study. Therefore, we changed the text by decreasing the tone and describing the necessity of mouse experiments to conclude about it (page 14-15).

9. *The paper suffers from relatively little detail given to describe many of the experiments.*

We increased description about experiments. But paper length limitation is a restriction.

10. *Better protein loading in Fig. 1C is needed to compare MAXER levels between tissues.*

This is a misunderstanding of the reviewer. It is Northern Blot but not WB. But it is true that control gene expression is variable among tissues, although the commercial filter should be loaded exactly with 2 microgram of messenger RNA. Thus, we further added actin as a control gene, which showed that equivalent amount of messenger RNA was loaded on each lane.

*11. This is an enormous paper with many figures, including supplemental. Many of the supplemental figures should be within the body of the paper, rather than supplemental. More judicious use of the figures would enhance the manuscript. For example, is the experiment shown in Supp. Fig. 9 really central to the paper?*

Previous Supplementary Figure 9 was deleted. Regarding integration of Supplementary Figures to Figures, since other reviewers suggested us to delete many figures, we consulted with the editor. We followed his advice in this revision.

*12. The description of the binding domain of MAXER with alpha-tubulin (Fig. 3E) should be better described within the text (p. 7). In addition, the description of the specific MAXER domain responsible for binding CDK5RAP3 is also needed in the text.*

Reviewer 3 and the editor recommended us to delete all the part about binding to tubulin, thus we will not describe it. We discussed about the homology of binding domain to CDK5RAP3 in the text (page 8).

Referee #2 (Remarks to the Author):

*Via microarray assays, the authors found that Maxer, a novel protein, is downregulated in cultured SCA1 cells. They further found that Maxer is an ER associated protein and is expressed in Bergmann glia. Loss of Maxer inhibits the proliferation of glial cells as well as glutamate transporter GLAST, while overexpression of Maxer can rescue them. The studies were well done. Many approaches and experiments were nicely coordinated to generate interesting results. The normal function of Maxer appears to be characterized. However, there are two general issues about the role of Maxer in the pathogenesis of SCA1.*

We thank the reviewer for the kind evaluation of our manuscript.

*The first one is that, as Maxer is also expressed in neuronal cells such as Purkinje cells, it is unclear whether reduction of Maxer in neurons is sufficient to induce neurotoxicity or neurodegeneration. Knowing this would help evaluate the role of glial Maxer in SCA1. Also, more quantitative results about the loss of Bergmann glial cells and Maxer in SCA1 mouse brains need to be provided, such as western blotting that is more quantitative than fluorescent imaging.*

This is a critical comment. We analyzed expression of Maxer in Purkinje cells by in situ hybridization (Figure 1D, arrowhead). We newly added Calbindin/Maxer double immunostaining of cerebellar tissues (Supplementary Figure 2A). These results showed almost no expression in Purkinje cells. We also analyzed expression by qPCR. According to this expression in primary cerebellar neurons are below 10<sup>-3</sup> of that in primary glial cells (data not shown). These analyses may exclude cell autonomous effect of Maxer in Purkinje cells.

Regarding quantitative results about the loss of Bergmann glial cells in SCA1 mouse brains, we added WB of Sox2 with cerebellar tissues (Figure 6D) and of Maxer with cerebellar tissues (Figure 2A).

*The other issue is why mutant ataxin-1 decreases the transcription of Maxer.*

This is again a critical question. However, since we think it is beyond the scope of this study and it would be our next project, we consulted with the editor and he accepted our plan to discuss in the text (page 15).

*I think that if the authors could provide further insight into the above issues, the manuscript would be much more interesting.*

*Specific issues*

*1. The reduction of Maxer was found in cultured cells transfected or infected with mutant Ataxin-1. The authors need to provide clear evidence that Maxer is reduced in Ataxin-1 knock-in mouse brains via western blotting of several mouse brain samples. Glial marker protein should also be probed on the blots to verify that the equal amounts of glial cells were examined.*

We increased the number of mice used for WB (Figure 2A). As Bergmann glial marker, we added WB of Sox2. As shown, due to the decrease of Bergmann glia in number, Sox2 band becomes weaker in Atx-KI mice (Figure 2A). We did not equilibrate the Sox2 signal because it means we load more tissue samples in KI mice, and it is obviously artificial. In response to the concern of the reviewer, we instead showed the signal ratios between Sox2 and Maxer bands.

*2. Some important results were from cultured cells that were transfected with Maxer or its fusion protein with GFP. The paper would be strengthened if there were more in vivo evidence for the subcellular distribution of endogenous Maxer. For example, figure 3 needs to show the association of endogenous Maxer, but not transfected GFP-Maxer, with ER. GFP-Maxer's subcellular localization is not very convincing, as the image resolution is not high enough.*

We added immunocytochemical analysis of subcellular localization of Maxer with anti-Maxer antibody instead of GFP-Maxer (Figure 3D)

*3. The authors need to provide evidence via western blotting to show if there is decrease of SOX2 in the cerebellum tissue, which can verify the decrease of Bergmann glia in ataxin-1.*

We performed the proposed WB with Sox2 antibody (Figure 2A), which confirmed our previous results.

*4. The authors focus on GLAST for glutamate transporter expression. They should also examine GLT-1, which is also important for up-taking glutamate by glial cells.*

We added analyses of GLT1 in Supplementary Figure 7. All the results support our findings with GLAST.

Referee #3 (Remarks to the Author):

*The manuscript by Shiwaku et al describes the identification of a novel ER protein that is downregulated by mutant ataxin-1 and leads to negatively impacts the development and function of cerebellar Bergmann glia. The authors propose the hypothesis that the effect of mutant ataxin-1 expression on Bergmann glia contributes to the pathogenesis of SCA1. A very large amount of data is presented in this manuscript, making it very difficult to evaluate. Nevertheless, there are a number of important experiments presented here that if distilled down to those critical for the author's main points could be come a communication that would be very useful to other investigators in the polyglutamine neurodegeneration field.*

We thank the reviewer for critical review and kind comments.

*I would suggest a number of revisions to improve this manuscript.*

*1) The first and most critical point for the authors of this manuscript is to obtain assistance from an editor, experienced in writing in English, who can assist them in assuring their ideas are clearly communicated using well constructed scientific English.*

We asked a native scientist to edit this manuscript.



2) A number of figures, sub figures and supplemental figures are not needed for the author's to make the main points of their paper and simply add confusion for a reader trying to glean the importance of this protein to the pathogenesis of SCA1. In addition, some figures make points the author's point unconvincingly.

I would recommend removing Fig. 1b, Fig. 3, Fig. 4a and 4e while moving the rest of Fig. 4 to supplementary figures, reformatting Fig 5 into two figures and removing supplementary figs. 1, 2 and 4.

We really appreciate the reviewer's comment about restructuring of the figures. With most advices, we agree, while we would like to keep a minor part of figures suggested to be removed by the reviewer. Therefore, we consulted with the editor asked his opinion. As the result, we removed Figure 3D, E, F. Figure 4A, C are moved to Supplementary Figure 4. Figure 4B, D, E are moved to Figure 5

and combined with Figure 5I. Figure 5 was split into two figures following the comment of the reviewer, and previous Figure 5A-C are now Figure 4.

Supplementary Figure 1, 2, and 4 were removed as requested.

3) Figure 2D and 2E would be more convincing if the nuclear and cytosolic markers were switched (Maxer w/ Sox 2 and Ataxin 1 w/ GFAP) because it looks like the are imaging bleed-through fluorescence.

We showed Maxer w/ Sox 2 and Ataxin 1 w/ GFAP in Supplementary Figure 2.

4) While I believe Fig. 3 should be removed because it is not really relevant to the main point of the paper, the images in it are also unconvincing. The images in 3D and 3F do not make the point well at all. Most of the images picked to show the ER pattern do so convincingly, but the images picked to show the co-localization w/ tubulin have a completely different pattern of localization.

This suggests that there is a strong likelihood that one or the other images are impacted by bleed through fluorescence.

Figure 3D and 3F were removed as requested. We added single transfection images in figure 3A.

5) The FACS analysis showing % of cells in G1 in Fig.'s 4 and 5 are not convincing. Is this really significantly different? Information about positive and negative controls and number of experimental replicates would help.

We added quantitative analysis of G1 fraction percentage in 4 independent experiments (Figure 5H). We employed mock and non-silencing siRNA as negative control and cyclin D1 siRNA as positive control. We added the information in the text and legend.

6) The authors should discuss how it happened that they found this protein more clearly. The way it is presented it is not clear because it says they found it during a proteomic and genomic analysis of cerebellar neurons. Did they find it by proteomics, expression profiling or both? Which neurons were they studying? Cultured neurons? Were glia present in the cultures? If not, why do they think they found a glial protein in these studies?

We found this gene only in mRNA expression profiling by microarray. The sensitivity of proteomics is not sufficient for detecting a protein expressed in a minor population of cerebellar cells (Bergmann glia). We used the established method to culture cerebellar neurons from P7 mouse brain. Ara-C was added to the culture of course to prevent glial proliferation, whereas Ara-C cannot eliminate Bergmann glial cells. We described these things in the text (page 5).

7) The authors need to discuss the issue that mutant ataxin-1 clearly has cell autonomous toxicities in Purkinje neurons as well, since transgenic mice expressing the mutant protein under a Purkinje neuron specific promoter develop ataxia and Purkinje neuron degeneration. While Maxer may play a role in preventing normal Bergmann glia function and thus make the cerebellum more vulnerable to injury, Maxer is not expressed in Purkinje neurons. Thus, it is still questionable whether the role of

*Maxer is a major contributor to SCA1 pathogenesis or simply a concurrent finding. The clinical course of the disease (neurodegenerative, not neurodevelopmental) suggests that the finding they are reporting of abnormal development and proliferation of Bergmann glia may not be of clinical importance. The data should be presented in this context.*

We completely agree with this comment from the reviewer. We appreciate and also believe in cell-autonomous toxicities in Purkinje cells. We cannot complete the extent of contribution of Maxer to SCA1 pathology. The issue about clinical course might be difficult, but we agree it is still questionable. We described about these issues in the discussion (page 14).

2nd Editorial Decision

10 May 2010

Thank you for submitting your revised manuscript for our consideration. Some of the original referees have now assessed your responses to the initial criticisms, and I am happy to inform you that there appear to be no more major objections towards publication of the paper in The EMBO Journal, after a few editorial changes. Referee 2 criticizes the insufficient information on the transgenic mice used in the study, and referee 3 still feels that the abstract would need thorough revision both for clarity and language (ideally involving the assistance of a native English-speaking scientist). Furthermore, this referee suggests removing/shortening the sections in Results and Discussion dealing with MAXER on microtubules and in stem cell proliferation, which (as discussed before) may detract from the more deeply investigated main findings in this rather comprehensive study. Finally, we should consider a more appropriate title for the study, and (based on suggestions from the referees), I would like to propose something along the lines of "Role for suppression of the novel ER protein MAXER by mutant ataxin-1 in Bergman glia in non-cell autonomous toxicity".

I am therefore returning the study to you once more, kindly inviting you to incorporate these additional changes into the manuscript text and to return the final version to us at your earliest convenience. After that, we should be able to swiftly proceed with its formal acceptance and production.

Yours sincerely,

Editor  
The EMBO Journal

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Referee #2 :

The revision and new data have addressed many of my issues and also some major issues of other reviewers. However, the authors need to provide the information for transgenic mice used in their study (where they obtained etc).

Referee #3 :

The revised manuscript is significantly improved. However, it still needs some minor revisions.

1) While the use of English is improved in the body of the manuscript, the abstract is still barely understandable. It needs to be carefully revised for clarity.

2) The authors should remove paragraphs in the Results and Discussion sections referring to the ability of Maxer to bind microtubules and the role of Maxer in stem cell proliferation. The paper is poorly focused on multiple topics even without these divergences.

3) A clearer title would be: MAXER, a novel ER membrane protein, is suppressed by mutant Ataxin-1, leading to reduced glutamate transporter expression and number of Bergmann glia.

2nd Revision - authors' response

12 May 2010

Thank you very much for your very kind evaluation for our manuscript. We also appreciate very much the constructive comments from the reviewers. We improved the manuscript exactly following the comments from you and the reviewers, as described in the following point-by-point response.

Taking your advice, we made the title "Suppression of the novel ER protein MAXER by mutant ataxin-1 in Bergman glia contributes to non-cell autonomous toxicity". We hope it is acceptable for you.

#### REFEREE REPORTS:

Referee #2 :

*The revision and new data have addressed many of my issues and also some major issues of other reviewers. However, the authors need to provide the information for transgenic mice used in their study (where they obtained etc).*

We thank the reviewer for kind evaluation. Regarding transgenic mice, which we directly received from Professor Zoghbi, we had described the fact in acknowledgement in the previous version. In addition, we added some information about mutant ataxin1 knock-in mice in p9 and p19.

Referee #3 :

*The revised manuscript is significantly improved. However, it still needs some minor revisions.*

We thank the reviewer very much for the kind evaluation.

*1) While the use of English is improved in the body of the manuscript, the abstract is still barely understandable. It needs to be carefully revised for clarity.*

Our previous version including abstract had been checked by a native scientist, Dr. Sam Barclay (Imperial College London). However, with the advice of the reviewer, we asked him once again to edit the abstract. He added several changes to increase the clarity of the content.

*2) The authors should remove paragraphs in the Results and Discussion sections referring to the ability of Maxer to bind microtubules and the role of Maxer in stem cell proliferation. The paper is poorly focused on multiple topics even without these divergences.*

We removed the description about microtubules (page 7, page 13) and stem cell proliferation (page 12). Accordingly, we deleted Supplemental Figure 3F, G, Supplemental Figure 9 and Referee Only Figure. We also removed corresponding figure legends and references.

*3) A clearer title would be: MAXER, a novel ER membrane protein, is suppressed by mutant Ataxin-1, leading to reduced glutamate transporter expression and number of Bergmann glia.*

Taking the advice of the editor, we took the title "Suppression of the novel ER protein MAXER by mutant ataxin-1 in Bergman glia contributes to non-cell autonomous toxicity".

