Intermediate filament dysregulation in astrocytes in the human disease model of KLHL16 mutation in giant axonal neuropathy (GAN)

Rachel Battaglia, Maryam Faridounnia, Adriana Beltran, Jasmine Robinson, Karina Kinghorn, J. Ashley Ezzell, Diana Bharucha-Goebel, Carsten Bonnemann, Jodi Hooper, Puneet Opal, Thomas Bouldin, Diane Armao, and Natasha Snider

Corresponding author(s): Natasha Snider, University of North Carolina at Chapel Hill

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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.)

RE: Manuscript #E23-03-0094
TITLE: Intermediate filament dysregulation and astrocytopathy in the human disease model of KLHL16 mutation in Giant Axonal Neuropathy (GAN)

Dear Dr. Snider:

Your manuscript, entitled "Intermediate filament dysregulation and astrocytopathy in the human disease model of KLHL16 mutation in Giant Axonal Neuropathy (GAN)" has been seen by two reviewers, whose comments are attached.

As you will see, both reviewers found your data and novel methods important for the field, and your findings of significant interest. The second reviewer, however, raises several important points, concerns and edit suggestions listed in the report, that should help improve the study and conclusions after a revision. Especially you should make an effort to provide a better support for your model of mRNA export defects in GAN cells, and address GFAP/vimentin localization if possible. Further including a few additional quantifications, show split channels in some of the microscope panels, update methods for improved clarity will help support your data. Due to some of the differences between the patient cell lines and the few base-edited control cells that you describe, more caution is also advised in the interpretations and some conclusions.

In sum, we would be happy to consider a revised manuscript that satisfies the concerns of the reviewer. These changes can be performed relatively easily and follow directly from your current results. Therefore, we look forward to receiving your revised manuscript, together with a letter indicating the changes you have made and your point-by-point responses to the referees.

Sincerely,

Diana Toivola
Monitoring Editor
Molecular Biology of the Cell

Dear Dr. Snider,

The review of your manuscript, referenced above, is now complete. The Monitoring Editor has decided that your manuscript is not acceptable for publication at this time, but may be deemed acceptable after specific revisions are made, as described in the Monitoring Editor's decision letter above and the reviewer comments below.

A reminder: Please do not contact the Monitoring Editor directly regarding your manuscript. If you have any questions regarding the review process or the decision, please contact the MBoC Editorial Office (mboc@ascb.org).

When submitting your revision include a rebuttal letter that details, point-by-point, how the Monitoring Editor's and reviewers' comments have been addressed. (The file type for this letter must be "rebuttal letter"; do not include your response to the Monitoring Editor and reviewers in a "cover letter.") Please bear in mind that your rebuttal letter will be published with your paper if it is accepted, unless you have opted out of publishing the review history.

Authors are allowed 90 days to submit a revision. If this time period is inadequate, please contact us at mboc@ascb.org.

Revised manuscripts are assigned to the original Monitoring Editor whenever possible. However, special circumstances may preclude this. Also, revised manuscripts are often sent out for re-review, usually to the original reviewers when possible. The Monitoring Editor may solicit additional reviews if it is deemed necessary to render a completely informed decision.

In preparing your revised manuscript, please follow the instruction in the Information for Authors (www.molbiolcell.org/info-for-authors). In particular, to prepare for the possible acceptance of your revised manuscript, submit final, publication-quality figures with your revision as described.

To submit the rebuttal letter, revised manuscript, and figures, use this link: Link Not Available

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Thank you for submitting your manuscript to MBoC. We look forward to receiving your revised paper.

Sincerely,

Eric Baker
Journal Production Manager
MBoC Editorial Office
mbc@ascb.org

Reviewer #1 (Remarks to the Author):

This is a compelling, incredibly well-written study delving into the molecular features pertaining to the remodeling and aggregation of the intermediate filament (IF) cytoskeleton with Giant Axon Neuropathy (GAN). The Authors optimized an in vitro system based on patient-derived iPSCs de-differentiated from skin fibroblasts obtained from seven different patients while utilizing CRISPR/Cas9 to create an isogenic control line. The resulting iPSCs were utilized to create neural progenitor cells, astrocytes, and brain organoids to test the fate of the IF expressed in each model system as a consequence of various mutations in the KLHL16 gene. One of the innovative aspects of the study is the focus on the contribution of astrocyte toxicity to diseases, using human cells. Indeed, this cell population plays a key role in several neurological disorders while its functions differ in rodents when compared to humans. GAN astrocytes and brain organoids exhibited marked perinuclear accumulation of the IF glial component GFAP as well as nuclear deformation. These results are in agreement with those obtained in other organ systems/diseases (e.g. laminopathies in cardiac muscle) and provide evidence that cytoskeletal instability is not only relevant in mechanically-challenged tissues and can result from their impaired proteostasis. The nuclear accumulation of KLHL16 mRNA suggests a novel mechanism of cellular dysfunction that amplifies the deleterious effects of haploinsufficiency. Intriguingly, the presence of vimentin exacerbated GFAP aggregation, possibly acting as a pro-aggregating seed. Overall, the study contributes a novel model system to address the molecular (e.g. translational and post-translational) mechanisms that cause IF aggregation and toxicity with human GAN, potentially opening new avenues for the creation of new therapies.

Reviewer #2 (Remarks to the Author):

Battaglia et al. establish induced pluripotent-derived stem cell (iPSC) models of giant axonal neuropathy (GAN), a fatal neurodegenerative disorder caused by mutations to the KLHL16/GAN gene, which encodes gigaxonin. Gigaxonin is a ubiquitin E3 ligase adaptor protein that regulates the levels of intermediate filament (IF) proteins in several tissues, including neurons and astrocytes. The authors created iPSCs from GAN patient skin fibroblasts and differentiated them into neural progenitor cells, astrocytes, and brain organoids, with or without CRISPR correction of the GAN disease-causing mutation to create isogenic control lines. These studies revealed that accumulation and aggregation of the IF protein vimentin may be an early and important event in causing the perinuclear aggregation of other proteins (e.g., glial fibrillary acidic protein [GFAP]) that is recapitulated in the authors' models and is a known feature of GAN and other neurodegenerative disorders, such as Alexander disease (AxD). The results also hint at a potential role for astrocytopathy in GAN pathophysiology.
Overall, the manuscript establishes elegant model systems that will be useful for studying GAN and other neurodegenerative disorders characterized by IF and other protein aggregation, complementing existing animal models. The establishment and validation of GAN brain organoids and iPSC-derived astrocytes are strengths. There are also clear phenotypes distinguishing GAN cells and a (single) isogenic edited control. Some of the authors' claims seem a bit too strong or speculative for the current data, so I think text revisions are warranted in several instances, detailed below. I recommend accepting the manuscript pending satisfactory revisions to address the below points.

Major comments

1. It is excellent that the authors used CRISPR to create controls where GAN patient iPSCs were base-edited to correct their KLHL16 mutations. In principle, these should be isogenic controls that are perfectly matched to the patient iPSCs but with wild type IF proteostasis. However, the authors performed base-correction on the cells of only two patients (2 and 7), and patient 7's cells are used almost exclusively for the experiments in the paper because the correction in the patient 2 line did not resolve GAN phenotypes. More caution is needed in interpreting the discordant results from so few base-edited lines. It would be unreasonable to demand the creation and testing of many more isogenic controls, given the experimental burden involved, but more conservative and thorough discussion of the current data would be helpful. For example, did the CRISPR correction of patient 2's cells restore gigaxonin expression? This point is not addressed. The authors also jump around a fair bit with which cells they use - Supp Figures 4/5 use patient 7 whereas Supp Figure 6 uses patient 2, even though both figures supposedly say something about gigaxonin function in astrocytes and even though we know there are relevant and important difference between these patients. The authors should provide a better rationale in the manuscript for why a given system was used in each experiment and add further consideration of potential caveats of the current isogenic control data to the Discussion section.

2. Quantitative and statistical analyses should be performed wherever possible. For example, the authors should provide

appropriate analyses for Figure 4G (nuclear lamin staining), Figure 5B, and Supp Figure 5A and 5C (ALDHL1 staining distribution looks abnormal in G332R cells, compared to isogenic controls, even if similar percentages of both populations are "positive" for ALDHL1 - is this true?). Provision of single-channel images, in addition to the merged images, in Supp Figure 5A and 5C would be informative.

3. Some claims regarding astrocytes in GAN seem inadequately supported by the current data. For example, regarding Figure 7A-D, the authors write: "These results suggest that the high level of expression and perinuclear accumulation of vimentin may be an important driver of astrocyte pathology in GAN, in part by facilitating GFAP aggregation." This seems like too big a leap, given that the results in question derive from SW13 cells, not astrocytes. In general, I wonder if the authors may be over-stating the importance of astrocytes in human GAN disease. Can they provide more evidence from the background literature to support their hypothesis that astrocytopathy contributes to disease progression or clinical manifestations in GAN patients/humans? Which features of the disease cannot be explained by neuronal dysfunction alone (i.e., what might astrocyte defects be doing)? Some additional discussion of these points would strengthen the manuscript.

4. I find the authors' model for mRNA export defects in GAN cells to be confusing and probably insufficiently supported by the current data. Regarding Figure 7E-G and the proposed model, is there a way to test the authors' claims that "IF aggregation may impede the nuclear transport and translation of KLHL16 to gigaxonin"? Is only KLHL16 mRNA sequestered or affected? What about other mRNAs? Why aren't other mRNAs tested in the FISH analyses? Actin looks normal on the Western (Figure 7C), so ACTB mRNA could presumably be used for a FISH control for normal nuclear export. Also, other positive control mRNAs are mentioned in the FISH methods section, but data with these are not shown or analyzed. If a specific effect on the nuclear retention of KLHL16 mRNA (but not other mRNAs) is proposed, could the authors elaborate on the potential mechanism? How would defective mRNA transport alone account for lower KLHL16 mRNA levels in mutant cells in the qPCR assays (since the qPCR protocol harvests total cellular mRNA, meaning the sum of whatever's in the nucleus and cytoplasm)? Also, in the Discussion, the authors write "The possibility that posttranscriptional mechanisms may contribute to GAN raises the question of whether GAN can be caused by mutations outside the KLHL16 coding region." I'm not sure how this notion fits the model that defective gigaxonin (i.e., mutated in the coding region) leads to vimentin/IF accumulation and thereby causes nuclear export defects for KLHL16 mRNA. Mutations in the noncoding regions of KLHL16 would make normal gigaxonin protein and presumably not lead to vimentin/IF accumulation or mRNA trafficking defects. Are the authors suggesting a splice site mutation or very low expression of wild type protein could disrupt mRNA processing and export? The bottom line is that the authors should provide a clearer and better supported explanation of their model of mRNA export defects in GAN cells.

Minor comments

1. Based on data in Figure 4 and elsewhere, the authors argue that aggregation of vimentin in GAN cells may promote GFAP mislocalization and aggregation, but vimentin and GFAP are not co-stained together in one experiment or image, making this assertion harder to evaluate. Do such data exist for GAN and isogenic control cells, and could they be added to Figure 4 to strengthen this central claim of the manuscript? Also, the lamin staining in Figure 4E, which is supposedly normal, is impossible to see in the merged image. Please consider providing single-channel panels in addition to the merged image and better annotate which panel is which (i.e., is the top or the bottom the isogenic in Figure 4E)? They don't look very different to me.

2. Some experiments could use better methods descriptions. For example, how many biological replicates were performed in Figure 1? How is the NF staining in Figure 5 done? Is this a single antibody that recognizes both NF-M and NF-H? Please provide the NF antibody information in the Methods. More experimental description about the assays in Supp Figure 1A, Supp 2E and 2F (e.g. reagents and kits used for these analyses) in the method section would be helpful.

3. The claims about similarities between AxD and GAN iPSC-astrocytes are intriguing but remain to be validated. It might be good to more clearly acknowledge in the text that these connections are speculative at the moment and merit future investigations.

4. In the Results section, the "RF" acronym for Rosenthal fibers is not defined upon first usage.

5. More consistent labeling of figures would be useful. For example, it would be helpful to add "isogenic" to "2D1" and "2D3" in Figures 2C and 3B, to match Figure 2B. It would also be good to clearly label the panels in Figure 6 (top = control and bottom = GAN).

6. Other KLHL gene expression data are in Figure 2E, not supplemental table 3 (which includes the qPCR primers), as the in-text callout indicates.

Rebuttal Letter
RE: Manuscript #E23-03-0094
TITLE: Intermediate filament dysregulation in astrocytes in the human disease model of KLHL16 mutation in giant axonal neuropathy (GAN)

We appreciate the Reviewers' suggestions and critiques, which we addressed in full by the inclusion of additional data and discussion. A point-by-point answer to reviewer comments is included below. Changes to the manuscript are tracked in blue font. The major additions we made to the figures are:

- Figure 2: added panels B and C
- Figure 3: added panel F
- Figure 4: added panel C
- Figure 8: added panel D
- Supplemental Figure 2: added panel I
- Supplemental Figure 5: added individual channels and additional ALDHL1 images
- Supplemental Figure 6: added individual channels and additional Lamin B1 images
- Supplemental Figure 7: new figure (supplement to data in Figure 5)
- Supplemental Figure 8: new figure (supplement to data in Figure 8)

Reviewer #1 (Remarks to the Author):

This is a compelling, incredibly well-written study delving into the molecular features pertaining to the remodeling and aggregation of the intermediate filament (IF) cytoskeleton with Giant Axon Neuropathy (GAN). The Authors optimized an in vitro system based on patient-derived iPSCs de-differentiated from skin fibroblasts obtained from seven different patients while utilizing CRISPR/Cas9 to create an isogenic control line. The resulting iPSCs were utilized to create neural progenitor cells, astrocytes, and brain organoids to test the fate of the IF expressed in each model system as a consequence of various mutations in the KLHL16 gene. One of the innovative aspects of the study is the focus on the contribution of astrocyte toxicity to diseases, using human cells. Indeed, this cell population plays a key role in several neurological disorders while its functions differ in rodents when compared to humans. GAN astrocytes and brain organoids exhibited marked perinuclear accumulation of the IF glial component GFAP as well as nuclear deformation. These results are in agreement with those obtained in other organ systems/diseases (e.g. laminopathies in cardiac muscle) and provide evidence that cytoskeletal instability is not only relevant in mechanically-challenged tissues and can result from their impaired proteostasis. The nuclear accumulation of KLHL16 mRNA suggests a novel mechanism of cellular dysfunction that amplifies the deleterious effects of haploinsufficiency. Intriguingly, the presence of vimentin exacerbated GFAP aggregation, possibly acting as a pro-aggregating seed. Overall, the study contributes a novel model system to address the molecular (e.g. translational and post-translational) mechanisms that cause IF aggregation and toxicity with human GAN, potentially opening new avenues for the creation of new therapies.

We are grateful for Reviewer 1's insightful comments and enthusiasm for our study. We agree that the effects of IF cytoskeleton instability impact multiple organ systems and diseases. New tools that can promote discovery of shared mechanisms of IF proteostasis will advance the field and we are think that our study will gain broad interest.

Reviewer #2 (Remarks to the Author):

Battaglia et al. establish induced pluripotent-derived stem cell (iPSC) models of giant axonal neuropathy (GAN), a fatal neurodegenerative disorder caused by mutations to the KLHL16/GAN gene, which encodes gigaxonin. Gigaxonin is a ubiquitin E3 ligase adaptor protein that regulates the levels of intermediate filament (IF) proteins in several tissues, including neurons and astrocytes. The authors created iPSCs from GAN patient skin fibroblasts and differentiated them into neural progenitor cells, astrocytes, and brain organoids, with or without CRISPR correction of the GAN disease-causing mutation to create isogenic control lines. These studies revealed that accumulation and aggregation of the IF protein vimentin may be an early and important event in causing the perinuclear aggregation of other proteins (e.g., glial fibrillary acidic protein [GFAP]) that is recapitulated in the authors' models and is a known feature of GAN and other neurodegenerative disorders, such as Alexander disease (AxD). The results also hint at a potential role for astrocytopathy in GAN pathophysiology.

Overall, the manuscript establishes elegant model systems that will be useful for studying GAN and other neurodegenerative disorders characterized by IF and other protein aggregation, complementing existing animal models. The establishment and validation of GAN brain organoids and iPSC-derived astrocytes are strengths. There are also clear phenotypes distinguishing GAN cells and a (single) isogenic edited control. Some of the authors' claims seem a bit too strong or speculative for the current data, so I think text revisions are warranted in several instances, detailed below. I recommend accepting the manuscript pending satisfactory revisions to address the below points.

We are grateful for Reviewer 2's comments, questions and suggestions, which helped us improve the paper. All comments have been addressed in full, as noted below.

Major comments

1. It is excellent that the authors used CRISPR to create controls where GAN patient iPSCs were base-edited to correct their KLHL16 mutations. In principle, these should be isogenic controls that are perfectly matched to the patient iPSCs but with wild type IF proteostasis. However, the authors performed base-correction on the cells of only two patients (2 and 7), and patient 7's cells are used almost exclusively for the experiments in the paper because the correction in the patient 2 line did not resolve GAN phenotypes. More caution is needed in interpreting the discordant results from so few base-edited lines. It would be unreasonable to demand the creation and testing of many more isogenic controls, given the experimental burden involved, but more conservative and thorough discussion of the current data would be helpful. For example, did the CRISPR correction of patient 2's cells restore gigaxonin expression? This point is not addressed. The authors also jump around a fair bit with which cells they use - Supp Figures 4/5 use patient 7 whereas Supp Figure 6 uses patient 2, even though both figures supposedly say something about gigaxonin function in astrocytes and even though we know there are relevant and important difference between these patients. The authors should provide a better rationale in the manuscript for why a given system was used in each experiment and add further consideration of potential caveats of the current isogenic control data to the Discussion section.

We tempered our language and provided a statement in the discussion regarding the limitation of the study. For our iPSC models GAN vs. control comparison, we used patient 7 cells throughout, as stated in the manuscript. Pertaining to patient 2 cells, we added new Supplemental Fig. 2I and removed Supplemental Fig.6 as we do not have the corresponding isogenic control for comparison. We state the reasons for using SW13 in the over-expression study in Figure 7 and GAN fibroblasts in the mRNA localization study in Figure 8.

2. Quantitative and statistical analyses should be performed wherever possible. For example, the authors should provide appropriate analyses for Figure 4G (nuclear lamin staining), Figure 5B, and Supp Figure 5A and 5C (ALDHL1 staining distribution looks abnormal in G332R cells, compared to isogenic controls, even if similar percentages of both populations are "positive" for ALDHL1 - is this true?). Provision of single-channel images, in addition to the merged images, in Supp Figure 5A and 5C would be informative.

This was addressed by addition of new Fig.4F; new Fig.5C; edited Supplemental Fig.5; new Supplemental Fig. 6; and new Supplemental Fig.7.

3. Some claims regarding astrocytes in GAN seem inadequately supported by the current data. For example, regarding Figure 7A-D, the authors write: "These results suggest that the high level of expression and perinuclear accumulation of vimentin may be an important driver of astrocyte pathology in GAN, in part by facilitating GFAP aggregation." This seems like too big a leap, given that the results in question derive from SW13 cells, not astrocytes. In general, I wonder if the authors may be over-stating the importance of astrocytes in human GAN disease. Can they provide more evidence from the background literature to support their hypothesis that astrocytopathy contributes to disease progression or clinical manifestations in GAN patients/humans? Which features of the disease cannot be explained by neuronal dysfunction alone (i.e., what might astrocyte defects be doing)? Some additional discussion of these points would strengthen the manuscript.

We moderated our language around the clinical significance and modified the statements regarding the conclusions drawn from Fig.7.

4. I find the authors' model for mRNA export defects in GAN cells to be confusing and probably insufficiently supported by the current data. Regarding Figure 7E-G and the proposed model, is there a way to test the authors' claims that "IF aggregation may impede the nuclear transport and translation of KLHL16 to gigaxonin"? Is only KLHL16 mRNA sequestered or affected? What about other mRNAs? Why aren't other mRNAs tested in the FISH analyses? Actin looks normal on the Western (Figure 7C), so ACTB mRNA could presumably be used for a FISH control for normal nuclear export. Also, other positive control mRNAs are mentioned in the FISH methods section, but data with these are not shown or analyzed. If a specific effect on the nuclear retention of KLHL16 mRNA (but not other mRNAs) is proposed, could the authors elaborate on the potential mechanism? How would defective mRNA transport alone account for lower KLHL16 mRNA levels in mutant cells in the qPCR assays (since the qPCR protocol harvests total cellular mRNA, meaning the sum of whatever's in the nucleus and cytoplasm)? Also, in the Discussion, the authors write "The possibility that posttranscriptional mechanisms may contribute to GAN raises the question of whether GAN can be caused by mutations outside the KLHL16 coding region." I'm not sure how this notion fits the model that defective gigaxonin (i.e., mutated in the coding region) leads to vimentin/IF accumulation and thereby causes nuclear export defects for KLHL16 mRNA. Mutations in the noncoding regions of KLHL16 would make normal gigaxonin protein and presumably not lead to vimentin/IF accumulation or mRNA trafficking defects. Are the authors suggesting a splice site mutation or very low expression of wild type protein could disrupt mRNA processing and export? The bottom line is that the authors should provide a clearer and better supported explanation of their model of mRNA export defects in GAN cells.

We added staining of control mRNAs (new Figure 8D and Supplemental Figure 8). We added the qPCR data on *KLHL16* total mRNA expression to Figure 2B-C. *KLHL16* mRNA levels do not differ between GAN and isogenic controls. We removed the working model panel and added more information on why we chose to examine *KLHL16* mRNA. Given the completely uncharacterized nature of the *KLHL16*

mRNA transcript and its exceptionally long 3'UTR region, we refrained from speculating about the downstream effects of nuclear sequestration but added more general discussion on this.

Minor comments

1. Based on data in Figure 4 and elsewhere, the authors argue that aggregation of vimentin in GAN cells may promote GFAP mislocalization and aggregation, but vimentin and GFAP are not co-stained together in one experiment or image, making this assertion harder to evaluate. Do such data exist for GAN and isogenic control cells, and could they be added to Figure 4 to strengthen this central claim of the manuscript? Also, the lamin staining in Figure 4E, which is supposedly normal, is impossible to see in the merged image. Please consider providing single-channel panels in addition to the merged image and better annotate which panel is which (i.e., is the top or the bottom the isogenic in Figure 4E)? They don't look very different to me.

Single-channel panels were added to Supplemental Figure 6. The vimentin-GFAP co-stains were only done with patient 2 (Y89S) cells. We do not have vimentin/GFAP co-stain the G332R and isogenic control cells and given the extended time period required to get GFAP+ iPSC-astrocytes, we are not able to generate those data in a reasonable time frame. We believe the total evidence presented supports our overall conclusions.

2. Some experiments could use better methods descriptions. For example, how many biological replicates were performed in Figure 1? How is the NF staining in Figure 5 done? Is this a single antibody that recognizes both NF-M and NF-H? Please provide the NF antibody information in the Methods. More experimental description about the assays in Supp Figure 1A, Supp 2E and 2F (e.g. reagents and kits used for these analyses) in the method section would be helpful.

Figure 1 (A,B) is from a single GAN patient. Details on NF antibody and the hPSC ScoreCard analysis of pluripotency were added to the Methods along with the key references.

3. The claims about similarities between AxD and GAN iPSC-astrocytes are intriguing but remain to be validated. It might be good to more clearly acknowledge in the text that these connections are speculative at the moment and merit future investigations.

We modified the text regarding this point.

4. In the Results section, the "RF" acronym for Rosenthal fibers is not defined upon first usage.

This is now defined.

5. More consistent labeling of figures would be useful. For example, it would be helpful to add "isogenic" to "2D1" and "2D3" in Figures 2C and 3B, to match Figure 2B. It would also be good to clearly label the panels in Figure 6 (top = control and bottom = GAN).

This was addressed.

6. Other KLHL gene expression data are in Figure 2E, not supplemental table 3 (which includes the qPCR primers), as the in-text callout indicates.

Thank you, we have addressed this in the text.

RE: Manuscript #E23-03-0094R
TITLE: "Intermediate filament dysregulation in astrocytes in the human disease model of KLHL16 mutation in giant axonal neuropathy (GAN)"

Dear Dr. Snider,

Thank you for submitting a well revised manuscript that I will be happy to accept for publication to MBoC.

I have a few minor requests for you to revise only related to clarity before final acceptance:

1. I was not able to locate the information from where the patient 1-7 biopsies/fibroblasts were obtained, could you clearly please include this information and any related possible consent information. Now only a short note is included for the one patient sample used in Fig. 1A and B.

2. It is a bit difficult to follow the iso-genic control cell line names/patient, and would ask you to clarify this, by adding the names of these control cell lines used, eg list them in the Materials and Method section, or to one of the tables (eg Fig. 1C table) to help the reader.

3 In the results last section, sentence 2 has an extra "that", and the sentence mid-section starting with "To adress this questions", needs editing for clarity.

Sincerely,
Diana Toivola
Monitoring Editor
Molecular Biology of the Cell

Dear Dr. Snider,

The review of your manuscript, referenced above, is now complete. The Monitoring Editor has decided that your manuscript requires minor revisions before it can be published in Molecular Biology of the Cell, as described in the Monitoring Editor's decision letter above and the reviewer comments (if any) below.

A reminder: Please do not contact the Monitoring Editor directly regarding your manuscript. If you have any questions regarding the review process or the decision, please contact the MBoC Editorial Office (mboc@ascb.org).

When submitting your revision include a rebuttal letter that details, point-by-point, how the Monitoring Editor's and reviewers' comments have been addressed. (The file type for this letter must be "rebuttal letter"; do not include your response to the Monitoring Editor and reviewers in a "cover letter.") Please bear in mind that your rebuttal letter will be published with your paper if it is accepted, unless you have opted out of publishing the review history.

Authors are allowed 15 days to submit a revision. If this time period is inadequate, please contact us immediately at mboc@ascb.org.

In preparing your revised manuscript, please follow the instruction in the Information for Authors (www.molbiolcell.org/info-for-authors). In particular, to prepare for the possible acceptance of your revised manuscript, submit final, publication-quality figures with your revision as described.

To submit the rebuttal letter, revised version, and figures, please use this link (please enable cookies, or cut and paste URL):
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If your manuscript contains a Significance Statement, please make sure that it consists of three separate bullet points totaling a maximum of 100 words and addressing each of the following points (see https://www.molbiolcell.org/curation-tools):
First bullet: What is the background context? What gap in knowledge does this study addres?
Second bullet: What are the key findings? What is unique or new about the approach?
Third bullet: Why is this paper significant? How might it influence future research?

Thank you for submitting your manuscript to MBoC. Please do not hesitate to contact this office if you have any questions.

Sincerely,

Eric Baker
Journal Production Manager
MBoC Editorial Office
mbc@ascb.org

Reviewer #2 (Remarks to the Author):

The revised manuscript has sufficiently addressed all comments regarding additional data, conclusions, and discussion. Specifically, they thoroughly characterized the CRISPR corrected cell lines in terms of gigaxonin protein expression, updated their statistical analyses with corresponding supporting imaging data, extended their discussion on the clinical significance of their findings, and revised their proposed model with additional data. For these reasons, we are satisfied with the authors' efforts and recommend acceptance for publication. Minor note: The authors' response says that they have added a Figure 3F, but I only see Figure 3A-C, so I believe they are referring to Figure 3C.

RE: Manuscript #E23-03-0094RR
TITLE: "Intermediate filament dysregulation in astrocytes in the human disease model of KLHL16 mutation in giant axonal neuropathy (GAN)"

Dear Natasha:

I am pleased to accept your manuscript for publication in Molecular Biology of the Cell.

Thank you for your clear edits, and congratulations for the acceptance of your manuscript to MBoC.

Sincerely,
Diana Toivola
Monitoring Editor
Molecular Biology of the Cell

Dear Dr. Snider:

Congratulations on the acceptance of your manuscript! Thank you for choosing to publish your work in Molecular Biology of the Cell (MBoC).

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We look forward to publishing your paper in MBoC.
Sign up for MBoC's new content alerts here https://tinyurl.com/3bcsyk3e

Sincerely,

Eric Baker
Journal Production Manager
MBoC Editorial Office
mbc@ascb.org
