nature portfolio

Peer Review File

Loss of symmetric cell division of apical neural progenitors drives DENND5A-related developmental and epileptic encephalopathy.

REVIEWER COMMENTS

Reviewer #1 (Remarks to the Author):

I have restricted my comments to the clinical and neuroradiological aspects of this paper, because these are my areas of expertise.

This paper presents clinical data for 23 individuals with biallelic variants (pathogenic, likely pathogenic or VUS) in DENND5A, representing the largest case series of this condition to date. The authors have collected a large amount of information via a clinician questionnaire, presented as raw data in Supplementary Table 1. This is valuable information, however in its current form it is not useful or interpretable. The authors should summarise this information is a systematic way across the case group, to provide a clear picture of which features occur commonly and which occur infrequently. They should use HPO terminology, for high level and more specific phenotypes.

Currently, the clinical summary in Extended Data Table 1 focuses on Seizures and Developmental milestones. Seizure types are not listed. Developmental information is provided in a non-systematic way within the table, which does not enable an objective assessment of the scope and severity of cognitive and behavioural impairments within the group. the age of participants is not accounted for in judging the severity of developmental delay.

Given the large amount of clinical data collected, authors could consider quantifying aspects of neurological and developmental phenotype to objectively assess the severity and extent of clinical impairments across cases and in relation to MRI findings and functional studies.

A major issue with this paper is that it claims to have identified a unique and distinctive neuroradiological signature associated with DENND5A variants. As currently presented, this claim is not justified. Extended Data Table 1 clearly shows that the range of neuroanatomical abnormalities within the case group is highly variable. No feature is consistently present across the group. Each individual feature is highly non-specific. Only 5 individuals with the group have a large number of neuroanatomical abnormalities, and the collection of abnormalities are unique to each individual. Whilst microcephaly is initially emphasised as a characteristic feature, the range of OFCs is from very small to very large.

Crucially, to claim that these combinations of severe structural abnormalities are distinct from other early abnormalities of CNS embryogenesis would require direct comparison with scans from other monogenic conditions, rated blind to genetic diagnosis by several paediatric

neuroradiologists. Currently, the claim is only made in relation to a highly selective literature-based discussion of a small number of other monogenic conditions (and even then, phenotypic overlaps with DENND5A are noted, contradicting the authors' main claim). If the authors think the neuroanatomical abnormalities of this condition really are distinctive, and relate to the specific mechanisms they have investigated, then they need to carry out an empirical study to show this.

The Discussion section of the paper is very short and does not mention the clinical aspects of the paper (either the findings or their limitations) and does not integrate the clinical data with the mechanistic studies.

I encourage the authors to revise these aspects of their paper, in order to make them more clinically and scientifically valid and useful.

Reviewer #2 (Remarks to the Author):

In this elegant study, Banks et al present data from the largest known cohort of patients Developmental and Epileptic Encephalopathies (DEE) associated with biallelic variants in DENND5A. DENND5A is a guanine nucleotide exchange factor and the molecular mechanisms by which DENND5A variants result in DEE is not well understood. The authors provide a comprehensive clinical description of 23 patients with DENND5A associated DEE, adding valuable clinical information relating phenotypic presentation of the disease as it relates to various biallelic DENNDA5A variants (including recommendations for updating some variant classifications to pathogenic). The authors then use a combination of human iPSC modeling, mouse, and zebrafish models to functionally characterize the various DENND5A variants. The protein characterization studies were well done, adding knowledge about the function of the protein, and revealing important protein-protein interactions of DENND5A with MUPP1 and PALS1. Similarly, studying DENNDA5A deficiency in human and model systems uncovered additional functions for this critical protein in neurodevelopment. While my enthusiasm for this for this study remain high, I do have a number of major and minor reservations that if addressed would provide for an excellent contribution to Nature Communications.

Main Critiques:

1. In the beginning of the article, it is unclear why the first section is entitled “main text”. As the first section of an article should be an introduction that contain background/summary information of relevant literature, there are a few sentences that appear out of context. For example, the sentence beginning: “ we now determine that DENND5A interacts with MUPP1/PALS..is a new result of this study and should be placed in the results section. Additionally, more background information

regarding earlier studies on DENND5A should be included in the first paragraph. Lastly, the authors cite several studies of genes related to cell division and polarity that are associated with DEE, but do not provide any specific information. It would strengthen the article to include specific examples of other genes associated with DEE and potential roles in centrosomal alignment during apical neural progenitor cell division.

2. The DENND5A KO iPSC model is an important control in many of the assays performed, although there are no specific experimental details how this KO was generated and which exons of DENND5A were targeted.

3. It is unclear what ages of mice were used for the experiments performed in Figures 3 and 5. Also, please include lower power images of panel D for Figure 3 (ie to what extent are there increased levels of NeuN+ cells?). Lower power images would also confirm staining is performed in the same anatomical plane in WT and KI mice. Similarly, based on this finding, there should be a depletion of neural progenitor cells in KI mice. Additional staining for NPC markers such as SOX2 should confirm this possibility.

4. Though the authors have attempted to characterize the localization of DENND5A in vitro and in vivo using IF techniques with commercial antibodies without success, it would be useful to attempt IF on NPCs transfected with Flag-tagged DENND5A WT and possibly also test some informative and stable DENND5A variants in vitro. There are several commercial Flag antibodies that work well for IF. These studies could potentially provide relevant mechanistic information on DENND5A function.

Minor Critiques:

1. Figure 1a-c should contain color, in particular in relation to Fig.1a as it is difficult to distinguish the black font from the dark grey font.

2. In the figure legend for Figure 1, participant 8 is described with no phenotype, although that observation is not consistent with Extended Data Table 1. Please check also participant 9.

3. “Supplementary Data 1” should be consistent with the labeling of “Extended Data Table 1”

4. It is unclear what the “X”s represent in Fig. 3d.

5. It is unclear what marker is used to define the dotted-line lumen in Figure 6b. Similarly, in determining the mitotic spindle angle, it is unclear what marker is used to identify the apical membrane.

Reviewer #1			
Reviewer Comment	**Rebuttal**	**New Data or Text/Justification**	**Changes/Figures**
"*The authors have collected a large amount of information via a clinician questionnaire, presented as raw data in Supplementary Table 1. This is valuable information, however in its current form it is not useful or interpretable. The authors should summarise this information is a systematic way across the case group, to provide a clear picture of which features occur commonly and which occur infrequently. They should use HPO terminology, for high level and more specific phenotypes.*"	We agree completely with the reviewer. Concisely summarizing the information using accurate and descriptive terminology that matches current convention is vital to conveying this highly valuable information.	We have re-contacted all of our clinical colleagues as described in detail in the next response. Based on this we have added text within the results section and changed some terms according to HPO terminology (e.g. "reduced volume" changed to "hypoplasia")	Figure 1b Paragraph 2 of Results, under subheading "Phenotypic characterization of individuals with biallelic DENND5A variants"
"Currently, the clinical summary in Extended Data Table 1 focuses on Seizures and Developmental milestones. Seizure types are not listed. Developmental information is provided in a non-systematic way within the table, which does not enable an objective assessment of the scope and severity of cognitive and behavioural impairments within the group. the age of participants is not accounted for in judging the severity of developmental delay."	We agree that the clinical summary table needed to be improved to be more useful and accurate. We very much thank the reviewer for this suggestion.	We have re-contacted all clinicians that completed our original questionnaires. All responded. From this we obtained more precise information on seizure types in accordance with up-to-date terminology set by the International League Against Epilepsy. Developmental outcomes are presented in a systematic way that accounts for participant age. We additionally included details on seizure drug resistance and medications to make the table more clinically useful.	Table 1 Paragraphs 3 and 5 of Results, under subheading "Phenotypic characterization of individuals with biallelic DENND5A variants"
"Given the large amount of clinical data collected, authors could consider quantifying aspects of neurological and developmental phenotype to objectively assess the severity and extent of clinical impairments across cases and in relation to MRI findings and functional studies."	We agree that presenting phenotypes in an objective and quantifiable manner would strengthen the study.	We have developed and implemented a scoring system for communication skills, motor skills, neurological abnormalities, and comorbidities to objectively assess the severity and extent of clinical impairments across the entire cohort. This includes consideration of DENND5A variant type and the presence or absence of microcephaly.	Table 1 Figure 1 e-g Paragraphs 5 and 6 of Results, under subheading "Phenotypic characterization of individuals with biallelic DENND5A variants"
"A major issue with this paper is that it claims to have identified a unique and distinctive neuroradiological signature associated with DENND5A variants. As currently presented, this claim is not justified. Extended Data Table 1 clearly shows that the range of neuroanatomical abnormalities within the case group is highly variable. No feature is consistently present across the group. Each individual feature is highly non-specific. Only 5 individuals with the group have a large number of	We understand that we cannot confidently make the claim that there is a unique neuroradiological signature associated with DENND5A variants based on our data and apologize for the statement. Only 5 complete scans were made available to us and were analyzed in detail, which is why only 5 individuals have a large number of abnormalities listed. The remaining neurological data come from clinical reports and questionnaires, resulting in a loss of detail. We agree with	We have modified the language in our text to reflect that the phenotype is interesting but that further study is required to establish whether there is a unique genotype-phenotype relationship.	Paragraph 7 of Results, under subheading "Phenotypic characterization of individuals with biallelic DENND5A variants" Paragraph 2 of Discussion

neuroanatomical abnormalities, and the collection of abnormalities are unique to each individual."	the reviewer that our original claim was an over-reach.		
"Crucially, to claim that these combinations of severe structural abnormalities are distinct from other early abnormalities of CNS embryogenesis would require direct comparison with scans from other monogenic conditions, rated blind to genetic diagnosis by several paediatric neuroradiologists. Currently, the claim is only made in relation to a highly selective literature-based discussion of a small number of other monogenic conditions (and even then, phenotypic overlaps with DENND5A are noted, contradicting the authors' main claim). If the authors think the neuroanatomical abnormalities of this condition really are distinctive, and relate to the specific mechanisms they have investigated, then they need to carry out an empirical study to show this."	We again apologize for claiming that our study shows a unique neurological phenotype associated with DENND5A variants. We did not and cannot bring in participants for a neuroimaging study due to geographical spread of participants. Only 5 complete scans were made available to us, which is insufficient to carry out a meaningful empirical study to definitively address this concern.	We have moved the literature-based discussion into the dedicated Discussion section of the paper, and have reiterated that further study would be required to determine whether there is an identifiable signature of DENND5A-related DEE.	Paragraph 2 of Discussion
"Whilst microcephaly is initially emphasised as a characteristic feature, the range of OFCs is from very small to very large."	Although the range of OFCs is very large, our data indicate that most participants fall below the third percentile, indicating that microcephaly is a key (albeit not universal) feature. Another case does not meet criteria for microcephaly, but is a borderline case whose OFC falls in the fourth percentile. One case of macrocephaly was mentioned, which we speculated could be due to this case's external hydrocephalus.	We have created a histogram to show the spread of known OFCs across the cohort to illustrate how most cases fall within the microcephalic range. We have also explained how even among the cases with normal OFCs, most had documented reductions in gray and/or white matter volume, suggesting that neurodevelopment could still be affected by our proposed cell division mechanism even in the absence of microcephaly.	Figure 1d Paragraph 4 of Results, under subheading "Phenotypic characterization of individuals with biallelic DENND5A variants" Paragraph 7 of Discussion
"The Discussion section of the paper is very short and does not mention the clinical aspects of the paper (either the findings or their limitations) and does not integrate the clinical data with the mechanistic studies."	We agree that a coherent integration between clinical data and mechanistic data is warranted.	We have added new commentary to the Discussion section in general, and added a figure that illustrates the relation between clinical findings (microcephaly and reduced gray matter volumes) with mechanistic findings.	Fig 7 Discussion

Reviewer #2			
Reviewer Comment	**Rebuttal**	**New Data or Text/Justification**	**Changes/Figures**
"In the beginning of the article, it is unclear why the first section is entitled "main text"."	This was a misunderstanding of Nature article formatting guidelines.	-	"Main Text" changed to "Introduction"
"As the first section of an article should be an introduction that contain background/summary information of relevant literature, there are a few sentences that appear out of context. For example, the sentence beginning: "we now determine that DENND5A interacts with MUPP1/PALS..is a new result of this study and should be placed in the results section."	We agree that the flow of the article needed improvement.	Several results-oriented sentences were removed from the introduction.	Paragraphs 1 and 4 of Introduction
"Additionally, more background information regarding earlier studies on DENND5A should be included in the first paragraph."	We agree that the article would be strengthened with more background information on DENND5A in the introduction.	We have added several sentences describing previous studies on DENND5A, which clarifies the rationale for our study.	Paragraph 1 of Introduction
"Lastly, the authors cite several studies of genes related to cell division and polarity that are associated with DEE, but do not provide any specific information. It would strengthen the article to include specific examples of other genes associated with DEE and potential roles in centrosomal alignment during apical neural progenitor cell division."	We agree that the inclusion of specific examples relating to DEE and cell polarity/division would strengthen the article.	We have added two examples of developmental genes where their roles in neural progenitor cell division is best defined.	Paragraph 2 of Introduction
"The DENND5A KO iPSC model is an important control in many of the assays performed, although there are no specific experimental details how this KO was generated and which exons of DENND5A were targeted."	This information was given in Methods under subheading "Establishment of cell lines", but we can briefly summarize it in the main text.	We have indicated the exon targeted where we introduce the cell line.	Paragraph 8 of Results, under subheading "Phenotypic characterization of individuals with biallelic DENND5A variants"
"It is unclear what ages of mice were used for the experiments performed in Figures 3 and 5."	We apologize for this oversight.	We have added the information in the Methods.	Methods, under subheadings "4-aminopyridine induced seizure assay" and "Immunohistochemistry"
"Also, please include lower power images of panel D for Figure 3 (ie to what extent are there increased levels of NeuN+ cells?). Lower power images would also confirm staining is performed in the same anatomical plane in WT and KI mice."	We agree that including lower power images is warranted.	We have added additional panels of 10X confocal images capturing as much as the lateral ventricles as possible, with the corpus callosum visible. The next panels in the figure are higher resolution images showing the regions indicated in the inset, as before. Note that the choroid plexus in KI animals are within a larger ventricle, so they were not captured in the same anatomical plane. Also note that we have observed differences in the thickness of the corpus	Figure 5d

		callosum, but variability was high and voxel-level analyses did not reach significance after correcting for multiple comparisons.	
"Similarly, based on this finding, there should be a depletion of neural progenitor cells in KI mice. Additional staining for NPC markers such as SOX2 should confirm this possibility."	We respectfully disagree on this point.	In the adult SVZ, "GFAP+ neural stem cells are the primary upstream source of new neurons in the SVZ of the intact and injured brain" (Williamson, Jones & Drew, 2019, doi: 10.1016/j.bbr.2019.112209). Since we did not observe a difference when quantifying GFAP+ cells in the SVZ after excluding the ependymal layer, it is unlikely that differences in more specific NPC markers would be observed. We have clarified the rationale for examining GFAP and not specialized NPC markers where we introduce the experiment in Results.	Paragraph 2 of Results, under subheading "Loss of DENND5A drives premature neuronal differentiation"
"Though the authors have attempted to characterize the localization of DENND5A in vitro and in vivo using IF techniques with commercial antibodies without success, it would be useful to attempt IF on NPCs transfected with Flag-tagged DENND5A WT and possibly also test some informative and stable DENND5A variants in vitro."	We agree that this is a reasonable experiment to gain insight on the subcellular function of DENND5A.	We expressed GFP-DENND5A WT and R710H in NPCs and confirmed, as hypothesized, that DENND5A is localized, in part, to centrosomes.	Figure 6d Paragraph 5 of Discussion
"Figure 1a-c should contain color, in particular in relation to Fig.1a as it is difficult to distinguish the black font from the dark grey font."	We agree that color will make the figure more easily interpretable.	We have color coded the variants in Fig. 1a. The pie chart was replaced with a color-coded Venn chart that we believe better demonstrates the extent of phenotypic overlap among cohort members. The bar graphs that were added to this figure also have color-coded individual data points.	Figure 1 a-b, d-g
"In the figure legend for Figure 1, participant 8 is described with no phenotype, although that observation is not consistent with Extended Data Table 1. Please check also participant 9."	We agree that this might be confusing. Also, we assume the reviewer meant participant 19 rather than 9.	We removed the legend, as it is no longer relevant to the updated figure. We meant to emphasize that these individuals do not have seizures and therefore cannot have DEE. We clarified their milder phenotypes in the text.	Paragraph 2 of Results, under subheading "Phenotypic characterization of individuals with biallelic DENND5A variants"
"Supplementary Data 1" should be consistent with the labeling of "Extended Data Table 1"	"Supplementary Data 1" was intended to present the raw data used for phenotypic analysis, and not to present additional results such as in our Extended Data tables and figures.	We moved this information into the Source Data file.	Source Data
"It is unclear what the "X"s represent in Fig. 3d."	We agree that the figure legends were lacking clarification.	We have indicated in the legends where these plots appear that the X's represent the mean.	Legends for figures 2d-f, 6b

"It is unclear what marker is used to define the dotted-line lumen in Figure 6b. Similarly, in determining the mitotic spindle angle, it is unclear what marker is used to identify the apical membrane."	We apologize that our description of the assay was missing this information.	We added this information in Methods, in our description of the assay in Results, and in the figure legend.	Methods, under subheading "Neural rosette formation assay" Paragraph 1 of Results, under subheading "Loss of DENND5A misorients mitotic spindles" Figure 6a legend

REVIEWER COMMENTS

Reviewer #1 (Remarks to the Author):

I am grateful to the authors for their clear and comprehensive revision of their paper. The increased quantity and structure of clinical data will increase the value of this work. I have some questions about presentation and interpretation of results:

1. Numerical scoring of clinical severity should be referenced in the Methods section, not just results.

2. It would be preferable to combine Figures 2 and Extended Data Figure 2 into a single figure. This would better illustrate the convergences and hetereogeneity in imaging findings. Even as currently displated, Figure 2 indicates that anatomical features are highly variable.

3. I remain concerned that the authors claim that there is a distinctive neuroradiological signature to this condition, without strong evidence for this and in the face of high variability. For example, page 11 line 280 describes a "complete phenotype" but this is present in only 2 / 24 cases. They should comment on lack of radiological clinical correlation, and lack of prediction from genotypes to radiology. I do not think the Discussion should emphasise (line 472) a "specific combination of features" without stronger evidence. Line 495 refers to an "interesing genotype-phenotype relationship" but it is not clear what relationship this is - generally this would require analysis within a disorder (which they have not completed for neuroimaging findings) and not differences between disorders (which I do not think they can strongly argue in view of heterogeneity and lack of data).

4. I also remain concerned about their commentary on 3 cases without seizures, for which they state "these individuals do not have DENND5A-related DEE and that one or more of these variants may be benign". There is ample precedent for individuals with pathogenic variants in DEE genes not having seizures (eg STXBP1 as a common example), and having variable neurodevelopmental disorder presentations. Seizures may present at a later age. The authors have not provided definitive functional studies which discriminate between these variants and other VUSs about which they are more confident. Hence I think the wording should be more cautious i.e. these variants may be pathogenic and clinical presentations represent continuous spectrum of severity.

5. Results section on expression levels needs a different sub-heading, as this is not part of phenotypic characterisation. This section is difficult to interpret, particularly because of the

variability in results of over-expression studies, which I think should be raised and discussed as a limitation since does not contribute to interpretation of VUS results or phenotypic variability.

6. The authors' present a disease model involving premature neuronal differentiation, because "KI mice have a significantly higher percentage of post-mitotic neurons expressing NeuN compared to WT" at a single time point. However, with extensive differences in neuronal proliferation also demonstrated, is this evidence strong enough to support this model, and their proposal of a shortened time window for proliferation versus differentiation? Are there other explanations for the result? What other experiments might the authors suggest carrying out in future to extend and challenge this interesting proposal?

7. Extended Data Figure 4 legend "c" should read "e".

Reviewer #2 (Remarks to the Author):

The authors have substantially improved the overall quality of this manuscript and also greatly improved the scientific rigor of this study. The authors sufficiently addressed all of my concerns and I highly recommend the publication of this manuscript without further revisions.

Reviewer #1			
Reviewer Comment	**Rebuttal**	**New Data or Text/Justification**	**Changes/Figures**
"Numerical scoring of clinical severity should be referenced in the Methods section, not just results."	We agree that phenotype severity scoring systems should be presented in the Methods and we apologize for the oversight.	We added a summary statement in the Method section and referenced Supplementary Methods for detailed scoring tables.	Methods, under subheading "Phenotypic data collection and analysis"
"It would be preferable to combine Figures 2 and Extended Data Figure 2 into a single figure. This would better illustrate the convergences and hetereogeneity in imaging findings. Even as currently displayed, Figure 2 indicates that anatomical features are highly variable."	We agree that including all images in one figure more accurately represents the spectrum of neuroanatomical features observed in individuals with biallelic *DENND5A* variants.	We combined both figures into one and edited the descriptive text in the legend and results to match. We also revised our wording to reflect the heterogenous and variable nature of imaging findings.	Figure 2 Figure 2 legend Results, paragraph 6 under subheading "Phenotypic characterization of individuals with biallelic DENND5A variants".
"I remain concerned that the authors claim that there is a distinctive neuroradiological signature to this condition, without strong evidence for this and in the face of high variability. For example, page 11 line 280 describes a "complete phenotype" but this is present in only 2/24 cases. They should comment on lack of radiological clinical correlation, and lack of prediction from genotypes to radiology."	We thank the reviewer for correcting our language to accurately reflect the data collected.	The two cases originally stated as having a "complete phenotype" are reworded to having "the most extensive neuroanatomical phenotypes" in the group. We also changed the language in the discussion. We emphasize that our literature-based comparison with other DEEs is based on the two cases with the most extensive phenotypes, that developmental and radiological phenotypes do not always correlate, and that the present study is insufficient to determine a genotype-phenotype relationship.	Paragraph 7 under subheading "Phenotypic characterization of individuals with biallelic DENND5A variants". Paragraph 1 of Discussion.
"I do not think the Discussion should emphasise (line 472) a "specific combination of features" without stronger evidence. Line 495 refers to an "interesting genotype-phenotype relationship" but it is not clear what relationship this is – generally this would require analysis within a disorder (which they have not completed for neuroimaging findings) and not differences between disorders (which I do not think they can strongly argue in view of heterogeneity and lack of data)."	We agree that our wording was too strong and have corrected it to reflect a more accurate overview of the phenotypes observed.	The word "specific" was deleted and the wording clarified that our literature-based discussion is based only on the phenotypes found in the most severe cases in the cohort. We also state that as is, our study cannot determine a genotype-phenotype relationship.	Paragraph 1 of Discussion.
"I also remain concerned about their commentary on 3 cases without seizures, for which they state "these individuals do not have DENND5A-related DEE and that one or more of these variants may be benign". There is ample precedent for individuals with pathogenic variants in DEE genes not	We again thank the reviewer for critically assessing our language to accurately present and discuss the data we have collected.	We have removed the statement of concern from the results section. We revised our discussion to say that "13% (3 cases) of our small cohort did not meet criteria for DEE or experience seizures at the time of data collection", implying that	Results, paragraph 2 under subheading "Phenotypic characterization of individuals with biallelic *DENND5A* variants" Paragraph 2 of Discussion.

having seizures (eg STXBP1 as a common example), and having variable neurodevelopmental disorder presentations. Seizures may present at a later age. The authors have not provided definitive functional studies which discriminate between these variants and other VUSs about which they are more confident. Hence I think the wording should be more cautious i.e. these variants may be pathogenic and clinical presentations represent continuous spectrum of severity."		seizures or DEE may develop later. We provided multiple possible interpretations for the variants found in the 3 cases that did not present with seizures at the time of data collection, and that further functional studies must be performed to determine the pathogenicity of these variants.	
"Results section on expression levels needs a different sub-heading, as this is not part of phenotypic characterisation. This section is difficult to interpret, particularly because of the variability in results of over-expression studies, which I think should be raised and discussed as a limitation since does not contribute to interpretation of VUS results or phenotypic variability."	We agree that separating these results from the phenotypic characterization is warranted, and that the limitations of overexpression experiments needed to be discussed.	A new subheading was added. A statement discussing the limitations of overexpression studies was added.	Subheading "DENND5A expression analysis" and the last two sentences within the section.
"The authors' present a disease model involving premature neuronal differentiation, because "KI mice have a significantly higher percentage of post-mitotic neurons expressing NeuN compared to WT" at a single time point. However, with extensive differences in neuronal proliferation also demonstrated, is this evidence strong enough to support this model, and their proposal of a shortened time window for proliferation versus differentiation? Are there other explanations for the result? What other experiments might the authors suggest carrying out in future to extend and challenge this interesting proposal?"	We believe that our evidence is indeed sufficient to propose a shortened time window of neurogenesis as a primary driving force behind DENND5A-DEE. We did not intend to base our model solely (or even largely) on our KI mouse results. The combination of decreased stem cell proliferation (NPC experiments), increased cell-intrinsic differentiation (NPC experiments), and increased differentiation due to cell-extrinsic factors (extrapolated from neural rosette experiments -- because cells lacking DENND5A divide away from and lose contact with the apical surface and thus the stem cell niche in the developing ventricle) all independently result in a shortened period of neurogenesis resulting in microcephaly. As DENND5A appears to affect all three of these mechanisms, we are confident in the validity of our model as it stands. However, we recognize that in some areas our model was unintentionally presented as fact. We also appreciate the suggestion to propose additional experiments to	We presented slightly more cautious wording in the final paragraph of the Introduction to clarify that the shortened period of neurogenesis is our model, and not a definitive result. We clarified in the discussion that our model is based on the synthesis of all of our data as well as known mechanisms for microcephaly mechanisms. We added another paragraph to the Discussion that proposes additional experiments to test the validity of our model in mice, and how the results of the experiments that would expand on our model could potentially impact physician decisions on choosing appropriate antiseizure medications for patients.	Final sentence in last paragraph of Introduction. Last two paragraphs of Discussion.

	strengthen or expand the model.		
“Extended Data Figure 4 legend "c" should read "e".”	We thank the reviewer for pointing out this error.	“c” changed to “e”. Because Extended Data Figure 2 was combined with Figure 2, this figure is now titled Extended Data Figure 2.	Extended Data Figure 2 legend.

REVIEWERS' COMMENTS

Reviewer #1 (Remarks to the Author):

It has been a pleasure to review this paper - thank you for clearly detailing your adjustments to the manuscript following review, and your rationale for modifications. I look forward to seeing this article in print, and future clinically-impactful work from this group.