Life Science Alliance

Biallelic structural variations within FGF12 detected by long-read sequencing in epilepsy

Sachiko Ohori, Akihiko Miyauchi, Hitoshi Osaka, Charles Lourenco, Naohiro Arakaki, Toru Sengoku, Kazuhiro Ogata, Rachel Honjo, Chong Kim, Satomi Mitsuhashi, Martin Frith, Rie Seyama, Naomi Tsuchida, Yuri Uchiyama, Eriko Koshimizu, Kohei Hamanaka, Kazuharu Misawa, Satoko Miyatake, Takeshi Mizuguchi, Kuniaki Saito, Atsushi Fujita, and Naomichi Matsumoto
DOI: https://doi.org/10.26508/lsa.202302025

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Review Timeline:

Submission Date:	2023-03-06
Editorial Decision:	2023-03-29
Revision Received:	2023-05-10
Editorial Decision:	2023-05-11
Revision Received:	2023-05-22
Accepted:	2023-05-23

Scientific Editor: Novella Guidi

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.)

March 29, 2023

Re: Life Science Alliance manuscript #LSA-2023-02025-T

Naomichi Matsumoto
Yokohama City University Graduate School of Medicine
Yokohama

Dear Dr. Matsumoto,

Thank you for submitting your manuscript entitled "Biallelic structural variations within FGF12 detected by long-read sequencing in epilepsy" to Life Science Alliance. The manuscript was assessed by expert reviewers, whose comments are appended to this letter. We invite you to submit a revised manuscript addressing the Reviewer comments.

To upload the revised version of your manuscript, please log in to your account: https://lsa.msubmit.net/cgi-bin/main.plex

You will be guided to complete the submission of your revised manuscript and to fill in all necessary information. Please get in touch in case you do not know or remember your login name.

While you are revising your manuscript, please also attend to the below editorial points to help expedite the publication of your manuscript. Please direct any editorial questions to the journal office.

The typical timeframe for revisions is three months. Please note that papers are generally considered through only one revision cycle, so strong support from the referees on the revised version is needed for acceptance.

When submitting the revision, please include a letter addressing the reviewers' comments point by point.

We hope that the comments below will prove constructive as your work progresses.

Thank you for this interesting contribution to Life Science Alliance. We are looking forward to receiving your revised manuscript.

Sincerely,

Novella Guidi, PhD
Scientific Editor
Life Science Alliance

A. THESE ITEMS ARE REQUIRED FOR REVISIONS

-- A letter addressing the reviewers' comments point by point.

-- An editable version of the final text (.DOC or .DOCX) is needed for copyediting (no PDFs).

-- High-resolution figure, supplementary figure and video files uploaded as individual files: See our detailed guidelines for preparing your production-ready images, https://www.life-science-alliance.org/authors

-- Summary blurb (enter in submission system): A short text summarizing in a single sentence the study (max. 200 characters including spaces). This text is used in conjunction with the titles of papers, hence should be informative and complementary to the title and running title. It should describe the context and significance of the findings for a general readership; it should be written in the present tense and refer to the work in the third person. Author names should not be mentioned.

-- By submitting a revision, you attest that you are aware of our payment policies found here: https://www.life-science-alliance.org/copyright-license-fee

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We encourage our authors to provide original source data, particularly uncropped/-processed electrophoretic blots and

spreadsheets for the main figures of the manuscript. If you would like to add source data, we would welcome one PDF/Excel-file per figure for this information. These files will be linked online as supplementary "Source Data" files.

IMPORTANT: It is Life Science Alliance policy that if requested, original data images must be made available. Failure to provide original images upon request will result in unavoidable delays in publication. Please ensure that you have access to all original microscopy and blot data images before submitting your revision.

Reviewer #1 (Comments to the Authors (Required)):

The authors here describe the discovery of two biallelic intragenic structural variations (SVs) in FGF12 gene identified by applying long-read whole genome sequencing (LRWGS) on two young patients with epileptic encephalopathy (DEE). To validate the molecular pathomechanisms of these biallelic FGF12 SVs/SNV, highly sensitive gene expression analyses using lymphoblastoid cells from the patient with biallelic SVs, structural considerations, and Drosophila in vivo functional analysis of the SNV were performed, confirming the loss-of-function. They conclude that LRWGS is important in identifying these mutations. The manuscript is of interest and needs only some improvement, mainly on the following aspects:
- I am curious about the presence of tremor/tremolousness in your patient(s). A recent systematic review has documented the presence of tremors in about 45% of cases studied using long-read sequencing (LRS) in neurodegenerative disorder. I was wondering if the authors could argue on these aspects, perhaps in the "The paper explained" section (See also Marsili L, Duque KR, Bode RL, Kauffman MA, Espay AJ. Uncovering Essential Tremor Genetics: The Promise of Long-Read Sequencing. Front Neurol. 2022 Mar 23;13:821189. doi: 10.3389/fneur.2022.821189).
- The authors mention about long-read whole genome sequencing (LRWGS) in the introduction, but what about LRS without WGS? Please, argue.
- I would expand the conclusions section a bit, better highlighting the next-steps related to these observations. Shall we use more frequently LRWGS in developmental epilepsy disorders (perhaps with clear family history)?

Minor comments;
- When describing the treatments with anti-epileptic drugs (AEDs) in the two patients, please add dosages of each AED.
- Page 7, Line 142: What does it mean "sometimes increased with fever"? Please, be more specific.
- Page 8, Line 162: " and at 6 years " please, add "of age".
- Page 10, Line 217: "Of the father" Please, add "of Patient 1"
- Page 23, Line 497: I believe it is "Escherichia Coli".

Reviewer #2 (Comments to the Authors (Required)):

This study reports for the first time biallelic intragenic SVs and a homozygous SNV in FGF12 in two patients with epilepsy by applying long-read whole genome sequencing. Expression analysis and in silico structural predictions of the interaction between FGF12 and NaV were done to support that epilepsy is caused by loss-of-function (LoF) due to biallelic FGF12 alterations. Moreover, in vivo functional analyses in Drosophila were performed.

The study is well conducted and reports novel findings, as heterozygous FGF12 mutations had previously been reported in DEE but never as biallelic mutations. This study further suggests a mechanism by LoF.

Comments
- Overall, the manuscript and figures should be shortened.
- The meaningful contribution on the part of Drosophila in vivo functional analysis is not very convincing and indirectly suggests a LoF of the missense mutation. It seems not to be essential to the manuscript. I, therefore, suggest removing it.
- Authors should further discuss to which extent the phenotype of patients with homozygous FGF12 mutations is more severe than those with heterozygous.

Comments from the reviewer(s):

Reviewer #1 (Comments to the Author):

The authors here describe the discovery of two biallelic intragenic structural variations (SVs) in FGF12 gene identified by applying long-read whole genome sequencing (LRWGS) on two young patients with epileptic encephalopathy (DEE). To validate the molecular pathomechanisms of these biallelic FGF12 SVs/SNV, highly sensitive gene expression analyses using lymphoblastoid cells from the patient with biallelic SVs, structural considerations, and Drosophila in vivo functional analysis of the SNV were performed, confirming the loss-of-function. They conclude that LRWGS is important in identifying these mutations.
The manuscript is of interest and needs only some improvement, mainly on the following aspects:

I am curious about the presence of tremor/tremolousness in your patient(s). A recent systematic review has documented the presence of tremors in about 45% of cases studied using long-read sequencing (LRS) in neurodegenerative disorder. I was wondering if the authors could argue on these aspects, perhaps in the "The paper explained" section (See also Marsili L, Duque KR, Bode RL, Kauffman MA, Espay AJ. Uncovering Essential Tremor Genetics: The Promise of Long-Read Sequencing. Front Neurol. 2022 Mar 23;13:821189. doi: 10.3389/fneur.2022.821189).

> Thank you very much for kindly evaluating our manuscript and providing us insightful comments.
Patient 2 manifested tremors, we therefore described them in the clinical features section, specifically on page 8, lines 167, 176, and Table 1. Patient 1 did not have tremors.
Based on the other reviewer comment, "The Paper Explained" section was removed to shorten the revised manuscript. Therefore, we instead added the above description in discussion section: page 17, lines 381-385. We hope this change is satisfactory to you.

The authors mention about long-read whole genome sequencing (LRWGS) in the introduction, but what about LRS without WGS? Please, argue.

> We mentioned “LRWGS” and “target-LRS” as well in the introduction section, page 5 lines 93-97.

- I would expand the conclusions section a bit, better highlighting the next-steps related to these observations. Shall we use more frequently LRWGS in developmental epilepsy disorders (perhaps with clear family history)?

> We have expounded upon our expectation toward LRS in the concluding section, specifically on page 18, lines 401-404.

Minor comments;

- When describing the treatments with anti-epileptic drugs (AEDs) in the two patients, please add dosages of each AED.

> We have added them.

- Page 7, Line 142: What does it mean "sometimes increased with fever"? Please, be more specific.

> Her seizures got more frequent during febrile episodes. (specifically on page 8, lines 156.)

- Page 8, Line 162: " and at 6 years " please, add "of age".

> Added (page 9, line 180).

- Page 10, Line 217: "Of the father" Please, add "of Patient 1"

> Added (page 11, lines 236).

- Page 23, Line 497: I believe it is "Escherichia Coli".

> Changed (page24, line 526).

Reviewer #2 (Comments to the Author):

This study reports for the first time biallelic intragenic SVs and a homozygous SNV in FGF12 in two patients with epilepsy by applying long-read whole genome sequencing. Expression analysis and in silico structural predictions of the interaction between FGF12 and NaV were done to support that epilepsy is caused by loss-of-function (LoF) due to biallelic FGF12 alterations. Moreover, in vivo functional analyses in Drosophila were performed.

The study is well conducted and reports novel findings, as heterozygous FGF12 mutations had previously been reported in DEE but never as biallelic mutations. This study further suggests a mechanism by LoF.

> Thank you very much for your favorable comments.

Comments

- Overall, the manuscript and figures should be shortened.

> We tried to shorten our revised manuscript: Figures 4-6 were merged to new Fig. 4. The initial part of the discussion and the section of 'The Paper Explained' were omitted to avoid the redundancy, and was removed.

- The meaningful contribution on the part of Drosophila in vivo functional analysis is not very convincing and indirectly suggests a LoF of the missense mutation. It seems not to be essential to the manuscript. I, therefore, suggest removing it.

>Thank you very much for your suggestion. As we believe it is still important to show the loss of function aspect by missense variants, we retain the Drosophila functional data in the revised manuscript but move it to the supplemental materials (Fig S5). We hope this change is acceptable.

- Authors should further discuss to which extent the phenotype of patients with homozygous FGF12 mutations is more severe than those with heterozygous.

> The clinical manifestations of patients with biallelic structural variations (SVs) or missense variant were further discussed based on the symptoms exhibited by previously reported patients carrying heterozygous aberrations in discussion section (page 17, lines 385-388).

May 11, 2023

RE: Life Science Alliance Manuscript #LSA-2023-02025-TR

Naomichi Matsumoto
Yokohama City University Graduate School of Medicine
Yokohama

Dear Dr. Matsumoto,

Thank you for submitting your revised manuscript entitled "Biallelic structural variations within FGF12 detected by long-read sequencing in epilepsy". We would be happy to publish your paper in Life Science Alliance pending final revisions necessary to meet our formatting guidelines.

Along with points mentioned below, please tend to the following:

-please upload the main and supplementary figures as single files
-please upload your video file as a single file that is not in a zip file format
-please add ORCID ID for both corresponding authors-you should have received instructions on how to do so

Figure Check:
-Figure 2 and Figure S5 need a scale bar
-Fig. 5B and Figure S1B first column looks like a possible duplicate. Please provide source data for both panels.

If you are planning a press release on your work, please inform us immediately to allow informing our production team and scheduling a release date.

LSA now encourages authors to provide a 30-60 second video where the study is briefly explained. We will use these videos on social media to promote the published paper and the presenting author (for examples, see https://twitter.com/LSAjournal/timelines/1437405065917124608). Corresponding or first-authors are welcome to submit the video. Please submit only one video per manuscript. The video can be emailed to contact@life-science-alliance.org

To upload the final version of your manuscript, please log in to your account: https://lsa.msubmit.net/cgi-bin/main.plex
You will be guided to complete the submission of your revised manuscript and to fill in all necessary information. Please get in touch in case you do not know or remember your login name.

To avoid unnecessary delays in the acceptance and publication of your paper, please read the following information carefully.

A. FINAL FILES:

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Submission of a paper that does not conform to Life Science Alliance guidelines will delay the acceptance of your manuscript.

It is Life Science Alliance policy that if requested, original data images must be made available to the editors. Failure to provide original images upon request will result in unavoidable delays in publication. Please ensure that you have access to all original data images prior to final submission.

The license to publish form must be signed before your manuscript can be sent to production. A link to the electronic license to publish form will be sent to the corresponding author only. Please take a moment to check your funder requirements.

Reviews, decision letters, and point-by-point responses associated with peer-review at Life Science Alliance will be published online, alongside the manuscript. If you do want to opt out of having the reviewer reports and your point-by-point responses displayed, please let us know immediately.

Thank you for your attention to these final processing requirements. Please revise and format the manuscript and upload materials within 7 days.

Thank you for this interesting contribution, we look forward to publishing your paper in Life Science Alliance.

Sincerely,

Novella Guidi, PhD
Scientific Editor
Life Science Alliance

Reviewer #1 (Comments to the Authors (Required)):

The authors have successfully addressed all my points. I do not have any further comments. Good job!

Thank you very much for your constructive comments.

With regards to Figure 2, we could put scales only in patient 1 but not in patient 2 due to unavailability of original MRI data within 7days in patient 2. We also added a comment in the legend why we could not put scales of MRI in patient.

We also uploaded the main and supplementary figures as separated single files.

The images of the gels presented in Figure 5B and Figure S1B are actually different, representing RT-PCR and genomic PCR, respectively. We uploaded source data of both figures.

May 23, 2023

RE: Life Science Alliance Manuscript #LSA-2023-02025-TRR

Naomichi Matsumoto
Yokohama City University Graduate School of Medicine
3-9 Fukuura, Kanazawa-ku
Yokohama 236-0004
Japan

Dear Dr. Matsumoto,

Thank you for submitting your Research Article entitled "Biallelic structural variations within FGF12 detected by long-read sequencing in epilepsy". It is a pleasure to let you know that your manuscript is now accepted for publication in Life Science Alliance. Congratulations on this interesting work.

The final published version of your manuscript will be deposited by us to PubMed Central upon online publication.

Your manuscript will now progress through copyediting and proofing. It is journal policy that authors provide original data upon request.

Reviews, decision letters, and point-by-point responses associated with peer-review at Life Science Alliance will be published online, alongside the manuscript. If you do want to opt out of having the reviewer reports and your point-by-point responses displayed, please let us know immediately.

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Scheduling details will be available from our production department. You will receive proofs shortly before the publication date. Only essential corrections can be made at the proof stage so if there are any minor final changes you wish to make to the manuscript, please let the journal office know now.

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Again, congratulations on a very nice paper. I hope you found the review process to be constructive and are pleased with how the manuscript was handled editorially. We look forward to future exciting submissions from your lab.

Sincerely,

Novella Guidi, PhD
Scientific Editor
Life Science Alliance