Author’s response to reviews

Title: Molecular diagnosis in recessive pediatric neurogenetic disease can help reduce disease recurrence in families

Authors:
Mahmoud Issa (myissa2002@hotmail.com)
Zinayida Schlachetzki (z.schlachetzki@gmail.com)
Valentina Stanley (vstanley@ucsd.edu)
Renee George (reneegeorge@gmail.com)
Jennifer McEvoy-Venneri (jmcevoyvenneri@ucsd.edu)
Denice Belandres (dbelandres@ucsd.edu)
Hasnaa Elbendary (hasnaa_mohamed24@yahoo.com)
Khaled Gaber (krgaber@gmail.com)
Ahmed Nabil (ahmadnabil248@yahoo.com)
Mohamed Abdel-Hamid (mohamadnrc@hotmail.com)
Maha Zaki (dr_mahazaki@yahoo.com)
Joseph Gleeson (jogleeson@ucsd.edu)

Version: 1 Date: 08 Oct 2019

Author’s response to reviews:

MGNM-D-19-00221R2 October 8, 2019
Molecular diagnosis in recessive pediatric neurogenetic disease can help reduce disease recurrence in families

We would like to thank MGNM for the decision to move forward with our publication, and the opportunity to address reviewer comments. The first reviewer remains skeptical, and offers constructive criticism to improve the manuscript by clarifying precisely how many patients were studied and potentially benefited from the study. We have done all we can with this retrospective study design to address the first reviewer’s comments in order to improve the manuscript based upon constructive criticism. We tried to expressly detail the rational for our study design and the limitations of interpretation to just the 101 pregnancies in molecularly diagnosed families. The second reviewer is very positive and offers several suggestions to improve the manuscript, all of which we have followed. We address the peer review comments on a point-by-point basis.
Response to reviewers

First reviewer (Reviewer 1, previous Reviewer 2)

General comments

1. My main concern remains the numbers used for analysis. The authors find that NGS diagnosis leads to a significant reduction in recurrence, but this is by selecting the analysis to &lt;10% of the original cohort, who a) had a diagnosis and b) returned for parental counselling with a subsequent pregnancy. This is equivalent to performing a clinical trial and analyzing only those patients who have responded to the intervention. To then make a statement about the success of the intervention is meaningless! For this reason, in a clinical trial, analysis is performed on an intention-to-treat basis. In this case, it would mean using all 1172 families that were initially enrolled as denominator. I understand that this is difficult to do, as the authors do not know about the pregnancy rate in those that did not return. This needs to be discussed.
Response. We apologize for not being clearer about which subjects were being considered to support our finding of the benefit of molecular diagnosis in reducing recessive neurogenetic disease recurrence. Although our study started with 1172 families that underwent NGS diagnostics, the benefit (i.e. reduction of recurrence) was measured only in the 101 pregnancies that returned to clinic. We have modified the abstract and discussion to clarify this important point. We were not able to assess the potential benefits to the full cohort of 1172 families of enrolling in the study, nor to any of the 526 families that received a molecular diagnosis but did not return to clinic with a subsequent pregnancy. Nevertheless, we think that it is useful for the reader to learn about the overall study design, rather than limiting the manuscript to just the 91 families. The conclusions have now been tempered with careful attention to this criticism. The abstract now reads “Among the 101 pregnancies, disease recurrence in living offspring deviated from the expected 25% to the observed 12% ([95% CI 0·04 to 0·20], p=0·011). “ Furthermore, if requested, we could remove text referencing the larger 1172 or 526 cohort, and just focus on the 91 families that returned to clinic with a subsequent pregnancy.

2. Supposedly, estimations can be made about average fertility rates about the number of expected pregnancies. For instance, if one assumes the 91 families who were used for analysis are representative of the entire cohort, then there would have been a total of 1172 x 101/91 =1287 pregnancies in the observation period, of which 25%≈322 would have been likely affected. The termination of 16 of these thus reduces the number of affected siblings by &lt;5%. Is that statistically significant?
Response. We agree with Reviewer’s calculations. We have now better justified why we limited the assessment of benefit to only the subset of the cohort that had a molecular diagnosis and returned to clinic with a subsequent pregnancy. The conclusion from the abstract limits the interpretation of benefit to those with molecular diagnosis in an older child coupled with prenatal fetal genotyping.
Requested revisions

1. I do not accept the answer the authors have provided to my previous comment in that regard that “the recurrence rate even in families in whom no genetic diagnosis could be made might be less than the predicted 25%”. That’s precisely the point! Parent may make decisions on either not becoming pregnant again or using alternative diagnostic methods and that’s exactly why using a ‘historic 25%’ as comparison is meaningless and biasing the results in favor of the authors’ hypothesis.

Response. The Reviewer correctly points out that a prospective trial to study the potential impact of prenatal diagnosis could offer information beyond what our conclusions offer. But even the study design proposed by the reviewer could have inherent flaws, for instance, should such a study use naïve families that have never undergone genetic testing, families that have negative genetic testing, families that were provided a molecular diagnosis but that did not opt for fetal molecular testing? For these reasons, we suggest that the historic 25% recurrence risk is a valid benchmark. Future studies may be able to utilize alternative designs, such as comparing molecular testing with other modalities such as fetal imaging, where the recurrence for both may be below the historic 25%.

Reviewer 3 (Reviewer 2)

1. General comments: My overall impression is that the manuscript is sound and that the revision was done well, and took into account all the suggestions of the initial reviewers. I think that the manuscript improved in the process.

I agree with the suggestion to merge Table 1 with Supl Table 1, they provide very similar information, and presenting twice is overkill. I would consider it typical (important ) supplemental information.

Response. We appreciate this comment, and have now merged Suppl Table 1 and Main Table 1 into Suppl Table 1.

2. The authors speak about genes/variants that were re-interpreted as pathogenic, and as a consequence families were re-enrolled in the study p12 lines 5-7. In figure 2 they give the amount of families that concerns this. This is important information and should be provided in the text on p12 with a reference to figure 1 for clarity.

Response. Done

3. I found it interesting that in 7% of the cases WGS led to identification of the disease that was missed by exome. This is actually results material and could be expanded upon if needs. Now it is buried in the methods sections.

Response. We have now moved this section to the main text.

4. The study design section is quite long and could do with a bit of trimming as it re-states some information present in the introduction.

Response. Done

5. p4, early fetal ultrasound. What is exactly early? The authors should give numbers here, as this varies by country. The sentence on p4 line 13 about brain development needs a reference.

Response. Done
6. One important point that is unclear at the moment is what happened to the remaining 417 families. The results section on p13 line 1-2 I read that these were followed-up but didn’t have any pregnancies. However, in the discussion on p17 line 17-18 it says ’we were not able to follow up and determine if they were pregnant”. The authors need to sort out this important information.

Response. We followed up to the degree possible with a relatively short timeframe and now mention this in the results and discussion.