Reviewer's report

Title: Efficient strategy for the molecular diagnosis of intractable early-onset epilepsy using targeted gene sequencing

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Reviewer: Saadet Andrews

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Reviewer comments

Title: Efficient strategy for the molecular diagnosis of intractable early-onset epilepsy using targeted gene sequencing

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Manuscript number: MGNM-D-17-00074

Manuscript type: Research article

Authors: John Hoon Rim et al.

Rim et al reports targeted next generation sequencing of 172 genes. Their diagnostic yield is 37.8%.

1) Abstract reports 25 patients in 17 genes had pathogenic or likely pathogenic small nucleotide variants. They report 15 patients in 7 genes. These numbers are confusing. What are the other genes they identified and patient numbers, should be given.

2) Not clear where "small nucleotide variants" terminology is adapted from. Are those missense or truncating variants?
3) How is the diagnostic yield increased from 12.2% to 37.8%? Is this their previous study that the authors referring? Please clarify.

4) Patients are included with early onset epilepsy, but authors refer to West syndrome with higher diagnostic yield. Either remove the sentence or describe patient population in more details in material and methods as well as results.

5) Conclusion of abstract discuss stepwise and team-based approach, but there is no clarification in material methods and results what the authors used. Please clarify.

6) Manuscript uses variant and mutation. According to ACMG mutation is not used anymore. Please correct throughout.

7) Page 13, lines 16-21: Epileptic spasms are not very commonly used as seizure type. The percentages are not adding up with epileptic spasms, generalized and focal seizures. Please clarify.

8) There is a new International League Against Epilepsy classification paper replacing your reference#8 (Fisher-RS et al, Epilepsia, 58(4):522-530, 2017). Page 13, lines 31-36: Epilepsy syndromes are not used anymore.

9) Page 14, lines 13-23: It is not clear how the authors increased diagnostic yield from 12.2% to 20.3% with the review of the literature and identified 13 additional variants. Did they miss some of the candidate variants by bioinformatics? How can be diagnostic yield increased without looking at the phenotype, but reviewing the literature and looking at the data?

10) It is not clear how the authors reached increasing rate of diagnostic yield after completing of bioinformatics in their 3-steps. Please clarify.
11) Table 1: How is the variant changed category with step 3? Despite Richards et al reference is used for variant classification, it is not clear if the clinical data will be able to change variant classification. Please clarify.

12) Methods should include brief description of variant classification based on the Richards et al how the authors classified variants.

13) Page 14, lines 55,57: Listing the most common genes is unnecessary, only the most common gene STXBP1 should be given instead. Figure 2a is unnecessary, repeating the information from the table 1.

14) Table 1 should include parent test results, if those are de novo. Despite methods report this, but results do not mention.

15) Chromosomal microarray abnormalities do not report parent test results. The size is not important, if one of the parent has same microarray abnormality, it is called variants of unknown significance and cannot explain phenotype. Patent test results should be given in the text and table. Two duplications were previously reported, compare your patients with the patients reported in the literature in discussion.

16) Page 15, lines 16-18: What is the distinctive EEG pattern for Angelman syndrome?

17) Page 15, lines 21-38: Why do authors include this paragraph? What is the information the reader should receive? Is this paragraph related to genotype and phenotype? Paragraph introduced IS, assume this is infantile spasms. Do authors use West syndrome and IS together? Choose one, likely IS better, as west syndrome is quite old not used in the clinical practice anymore.

18) Page 15, lines 48-50: NGS abnormality positive and negative is not acceptable description. Please formulate for clarification.
19) Page 15, line 55: What is absence of eye contact? Did those patients developed eye contact and lost it? Or their development is severely delayed (<2-3 months of age) that they never developed eye contact. It is not clear what authors mean with that. Authors should also do same statistics for the developmental age for both group to compare.

20) Discussion has subtitles, likely unnecessary.

21) What is different in the study compared to the various previous similar targeted NGS studies? Did authors discover novel candidate genes?

22) Page 17, lines 28-50: It is not clear what authors are trying to report here. Do they think that the targeted panels should not include many genes? What is reported previously? Why did authors not apply WES instead of targeted NGS? Are other genes known epilepsy genes? At least SCN8A, SLC9A6, CHD2 are well known. Finding a single patient for those genetic disorders, does not make them other genes.

23) Page 18, section "Selection of appropriate patients": Do authors suggest that patients with epilepsy and GDD do not qualify for genetic testing? Genetic testing is not designed to determine the diagnostic rate or select patients according to increase diagnostic yield. It is designed to give patients a genetic diagnosis. Again, how the authors increase diagnostic yield after bioinformatics, is not clear.

24) Discussion requires a paragraph why the authors chose 172 genes. Their diagnostic yield is similar to the study they cited (reference#13), which included 17 genes in a larger patient enrolment.

25) WWOX patient has exon 6-8 duplication, all patients reported in the literature have deletions. How sure are the authors that this patient's genetic diagnosis is confirmed. Does the patient have similar phenotype to the patients reported in the literature?
Are the methods appropriate and well described?
If not, please specify what is required in your comments to the authors.

Yes

Does the work include the necessary controls?
If not, please specify which controls are required in your comments to the authors.

No

Are the conclusions drawn adequately supported by the data shown?
If not, please explain in your comments to the authors.

Yes

Are you able to assess any statistics in the manuscript or would you recommend an additional statistical review?
If an additional statistical review is recommended, please specify what aspects require further assessment in your comments to the editors.

Not relevant to this manuscript

Quality of written English
Please indicate the quality of language in the manuscript:

Acceptable

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