Reviewer's report

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

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Reviewer: Johannes Lemke

Reviewer's report:

The authors describe their stepwise approach in performing epilepsy panel diagnostics on a sample set of 74 EOE cases. They describe a diagnostic yield of 37.8% and emphasize their team-based approach for maximisation of results.

This is a nice cohort study with results worth to be published. However, I see several issues and I do not completely agree with the reported conclusions.

1) I strongly recommend validating pathogenicity of genetic variants according to the guidelines of the American College of Medical Genetics (Richards et al 2015) and not according to self-established inconsistent step-wise approaches.

2) The authors describe an increase of their diagnostic yield from 12.2 to 37.8%. However, I consider the described approach as quite prone to subjectivity and not in accordance to general diagnostic guidelines. 11 (12.2%) of variants were identified by pure bioinformatics. Another 13 variants were identified after literature research and in silico prediction. Another 9 variants were revealed by consensus discussions.

According to ACMG, many aspects of variant interpretations should be taken together and should not be counted individually. In my eyes, it does not make much sense to separate these aspects. E.g. a diagnostics report should never consists of a bioinformatics assessment or an in silico prediction alone - thus, the diagnostic yield results from the sum of all these criteria. Partial aspects (like bioinformatics, prediction, etc) alone cannot account as "diagnostic yield". Thus, there is no increase from 12.2 to 37.8%.

3) Introduction: In the first line, the authors describe EOE as a form of epilepsy that results in severe cognitive impairment. It is debatable to what extent cognitive impairment really is the result of epilepsy. I believe the authors rather wanted to refer to the fact that
EOE is often "associated with" in severe cognitive impairment. The phrase should be adapted accordingly.

4) Methods: the authors excluded cases with abnormal MRI or with metabolic abnormalities. Why? Several forms of EOE can be associated with structural brain lesions, such as abnormal gyration, agenesis of corpus callosum, etc. It seems plausible that haemorrhages and infarctions are excluded, but I do not see a reason why disease-associated malformations should be excluded, too. It is not comprehensible what MRI lesions led to exclusion from the study - as well as what metabolic abnormalities, and why. How many cases were excluded that way?

5) The authors report that Sanger pre-sequencing had often been performed. Is there a reason why the pre-sequencing especially contained MECP2?

6) Page 11, line 20: "The clinicians performed an in-depth review of each patient's phenotype and gave an opinion from their point of view." This approach is certainly helpful, but can also be misleading as it is biased towards the phenotypic spectrum that we (i.e. the clinicians) are aware of. It is better to rely on ACMG - see comments 1,2

7) Page 11, line 39: Parental samples were available in about how many of the cases?

8) Results: Apparently 51 of the 74 patients were diagnosed with West syndrome. Can the authors give an explanation for this rather high proportion?

9) Page 13, line 16: It is appears unlikely that an in silico prediction alone is capable to reveal 13 additional VUS in this cohort as being pathogenic. According to ACMG this is nearly impossible.

10) Especially the third step appears highly speculative and much less evidence-based than application of ACMG criteria.
11) Page 17, line 40: the authors describe an apparently pathogenic mutation in PRICKLE2. This is not an OMIM-associated disease gene and should be explained in more detail.

12) Page 18, line 1: The study from Carvill et al applied a 65-gene panel, but only 19/65 were diagnostic genes at that time. 46/65 were candidates. The yield of 10% is thus not suitable for comparison with diagnostic yields in this context.

13) Page 18, line 9-13: I agree. But should the total number of genes in a panel not usually contain the number of commonly mutated genes?

14) Page 19, line 1: the authors refer to "our high diagnostic yield" of 37.8%. In fact, this is a rather low yield. Other studies revealed a diagnostic yield in EOE of e.g. 57% (Møller, 2016, Segal 2016), 61% (Olson 2017)

I think all variants should be re-evaluated according ACMG (for each variant it should also be listed what criteria were fulfilled e.g. in a supplementary table). The description of the 3-step approach is dispensable. In my eyes, the finding of gen-phen correlations in the West syndrome cases (eye contact, severity) appears a lot more impressive.

**Are the methods appropriate and well described?**
If not, please specify what is required in your comments to the authors.

No

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

Unable to assess

**Are the conclusions drawn adequately supported by the data shown?**
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