Reviewer's report

Title: Targeted next generation sequencing with an extended gene panel does not impact variant detection in mitochondrial diseases

Version: 1 Date: 26 Jan 2018

Reviewer: Amit Rawat

Reviewer's report:

I commend the authors of the manuscript for their study and exhaustive research work including the detailed molecular and bioinformatics analyses they have conducted in different forms of Mitochondrial disease which are relevant both for pediatric as well adult patients. I have the following comments regarding the manuscript.

1. The title of the manuscript "Using larger panels does not improve diagnostic yield of mitochondrial disease which needs exome sequencing" is confusing. What are the larger panels? What are they intended for? They are surely NOT for increasing or improving the diagnostic yield of mitochondrial disease. The authors have included 80 patients diagnosed with mitochondrial disease based on clinical presentation, biochemical investigations and histological features and performed genetic analysis by analyzing defect in the mitochondrial DNA followed by a customized targeted next generation sequencing comprising of 281 genes which were enlisted in the NIH Gene Testing Registry at time of the study. Please change. A appropriate title could be "Targeted next generation sequencing with an extended gene panel does not impact variant detection in Mitochondrial diseases"

2. In the abstract section of the manuscript the authors mention Since the advent of next generation sequencing (NGS) and whole exome sequencing (WES), several studies have tried to evaluate the relevance of these methods for molecular diagnosis of mitochondrial diseases. The comparison between these different works is extremely difficult. Next generation sequencing and whole exome sequencing are NOT 2 different techniques. Whole exome sequencing is also a form of next generation sequencing. Comparison have NOT been made between next generation sequencing (NGS) and whole exome sequencing (WES) but between targeted gene sequencing using a panel of genes most relevant to the clinical phenotype vis a vis sequencing the whole coding region or the exome using NGS. Please change the word "works" to methodologies or techniques. The conclusions in the abstract section are NOT valid as the authors have NOT clearly show the utility of exome sequencing OVER and ABOVE the targeted sequencing in their study. The authors have not performed whole exome in any of 57 unresolved cases to prove conclusively the utility of whole exome sequencing over targeted NGS.
3. We sequenced a custom panel of genomic regions corresponding to 281 genes, selected in 2016 to be already involved in mitochondrial disorders. Please change "sequenced" to designed or synthesized.

4. Finally, although I do NOT contest the utility of a whole exome sequencing over a more, limited targeted sequencing in general. I have reservations about the same with regard to your study since you have NOT provided any experimental evidence whatsoever in your study to irrevocably demonstrate the utility of whole exome sequencing over a targeted NGS with a limited or extended panel of genes in mitochondrial disease.

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.

Yes

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

No

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:

Not suitable for publication unless extensively edited
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