Author’s response to reviews

Title: Exome sequencing of a patient with suspected mitochondrial disease reveals a likely multigenic etiology.

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Author’s response to reviews: see over
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RE: MS: 8564411098401832

Please find a revised version of the manuscript, “Exome sequencing of a patient with suspected mitochondrial disease reveals a likely multigenic etiology” that we would like you to consider for publication in *BMC Medical Genetics*. We have revised the manuscript to make it better comply with journal format and we have addressed the reviewer’s concerns.

If you have any questions, please contact me at pbonnen@bcm.edu.

Thank you,

Penelope E. Bonnen

Response to Editor’s Comments

The subject and his parents were consented for research and publication. We have included the following statement in the Methods section: “Informed consent was obtained from the subject and his parents for research and publication of clinical details according to a protocol approved by the Institutional Review Board for Baylor College of Medicine and Affiliated Hospitals.”

Response to Reviewers comments

We thank the reviewers for their helpful and insightful comments.
Reviewer Choi’s report:
Craigen et al performed an elegant analysis of a patient with presumably a mitochondrial defect but turned out to be a multitude of defects due to the combinatorial effects of at least two genes using whole exome sequencing. The manuscript is suitable for publication of the journal, however, a few improvements would make the story stronger.
- Table 1 shows summary of coding SNV and short indels detected from the patient. % of novel for indel calls are too high which implies that quality control of indel calling may have not done appropriately. Another evidence comes from novel het: novel hom ratio which is 158:30 which is quite different from the ratio of SNVs (647:38). I would suggest increasing threshold for indel calling cutoffs to remove possible false-positives.
- Perhaps the authors could display the full list of novel homozygous variants from the patient to convince the readers that the symptoms are not of mitochondrial origin.

Author’s response:
Table 1 shows the summary of SNVs and small indels including how many are considered ‘novel’. The criteria for being novel was to be absent from dbSNP132. We have now updated our analysis to using dbSNP137 and the results are in line with the reviewer’s expectations. Using dbSNP137 the ratio of novel het:hom SNVs is 0.056 and the ratio of novel het:hom InDels is 0.049 (previously it was 0.19). We have modified Table 1 to reflect this updated analysis.

We have created a new Table that lists the novel non-synonymous homozygous variants from the patient. It is now included in the manuscript as Table 2.

Reviewer Taylor’s report:
The authors describe an extremely interesting consanguineous family with a patient having clinical features of mitochondrial disease and cerebellar ataxia. The authors carried our exomic sequencing on the patient and both parents. They conclude that the presence of homozygosity for a SETX splice site mutation together with the presence of a missense mutation in the X-linked OCRL gene may have contributed to the patient’s clinical phenotype.

Minor essential revisions
1. That the SETX splice site mutation together with the presence of a missense mutation in the X-linked OCRL gene may have contributed to the patient’s clinical phenotype is plausible. I believe that the word likely’ should be inserted in the title to give “Exome sequencing of a patient with suspected mitochondrial disease reveals a likely multigenic etiology”.

Author’s response:
This change has been made in the revised text (please see tracked changes).

2. I am not aware of Senataxin 1 only Senataxin.
Author’s response:
This change has been made in the revised text (please see tracked changes).

3. The pedigree is helpful and interesting, but it would also be helpful to have the mutations in SETX and OCRL in a more accessible and more usual nomenclature using the coding sequence. Eg for SETX I think that it is SETX c.5375-1G>A.

Author’s response:
We have changed the mutation nomenclature in the revised text (please see tracked changes) and in Figure 1.

This begs the question, not answered here, of whether this mutation results in loss of exon 10 and loss of senataxin protein or expression of a truncated form of the protein. As exon 10 codes for 58aa, such a protein truncation would be detectable. 4. We have to assume that it was because the patient was not available for further clinical investigations (page 13) that these particular procedures were omitted. Both of these would require a cell line to be made from patient material (blood or skin biopsy). To mention this would be helpful.

Author’s response:
We have now included in the results section p11 mention that because the patient was lost to follow-up we were not able to evaluate him clinically for Lowe Syndrome and we were not able to obtain tissue from the patient to test if SETX c.5375-1G>A does in fact affect splicing or otherwise affect the protein (please see tracked changes).

5. I could not find an indication that serum AFP level was measured in the patient’s blood. The authors rather awkwardly describe the increased level of serum AFP in AOA2 patients as ‘variable increases’. Most would agree that the serum AFP level was invariably increased in AOA2 patients.

Author’s response:
We have removed the word ‘variable’ (please see tracked changes). Serum AFP was not measured in this patient.

Comment: The authors summary is correct, that exome sequencing improves the diagnostic ‘possibilities’ but that some patients will require further clinical and/or functional studies to achieve a complete diagnosis. This is the case here.