Peer Review File

Article information: http://dx.doi.org/10.21037/tp-20-167.

Reviewer A

The presentation of a previously-unreported mutation is valuable to the field. However, there are multiple mistakes in the text indicating a very low understanding of disease mechanism that must be addressed. The most problematic examples are:

1. Zellweger Spectrum Disorder (ZSD, not PBD) is caused by mutations in 13 different PEX genes. PBD is a broader disease umbrella that includes ZSD, Rhizomelic Chondrodysplasia Punctata, X-linked adrenoleukodystrophy, and peroxisomal single enzyme defects.

Reply: We thank you for your valuable suggestion, we have narrow the width of our study down to ZSD not PBD, throughout our manuscript.

2. PEX1 and PEX6 do not ubiquitinate PEX5; this is performed by PEX2, PEX10, and PEX12. PEX1 and PEX6 facilitate the removal of mono-ubiquitinated PEX5 from the peroxisome membrane so that it can be recycled for additional rounds of import. If PEX1 function is impaired, PEX5 cannot be recycled. There is no "PEX5 dependent peroxisomal protein importation machinery". PEX5 is the main cytosolic enzyme receptor that delivers peroxisomal enzymes to the peroxisome via the PEX13 and PEX14 importomer. Please refer to the many available reviews and figures of peroxisome biogenesis and import available in the literature (such as https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5678237/)

Reply: Thank you very much, we made changes accordingly in our revised manuscript (Lines 45-50, highlighted in yellow).

3. What are "rapidly accumulating PEX1 mutations"? Do mean more recently identified?

Reply: We would like to indicated uncommon mutations in PEX1 gene by the sentence, we have modified our manuscript (Lines 59-60, highlighted in yellow).

4. The current term is for your patient is "Severe ZSD", not "Zellweger Syndrome".

Reply: Thank you very much, we made changes accordingly throughout out manuscript.

5. Though disease presentation in patients with the common p.G834D mutation is indeed milder than most other missense mutations, this is not always true. An important example are the mutations causing PEX1-mediated "Heimler Syndrome" (the mildest form of ZSD).

Reply: Thank you very much. We have added your suggestions accordingly in our manuscript (Lines 45-46, highlighted in yellow)

6. The authors state that "Increased protein instability in higher temperature are among the underlying mechanism for PBD." This cannot be a cause for PBD, as the patients are not at 40 degrees C. This observation in patient cell lines together with the observation that PEX1-G843D function improves at lower temperatures, and that the protein is degraded, suggests that this specific missense protein is unstable. The cause for PBD is peroxisome dysfunction.

Reply: Thank you very much and we agree, we have removed redundant words in the sentence.

7. The truncating mutations you discuss typically result in NO PEX1 protein production. This is the reason for a more severe phenotype. The exception to this is a stop codon in the last 2 exons, which still allow for translation of the mRNA transcript. Missense and other mutations result in the production and presence of subfunctional (usually decreased) protein.

Reply: Thank you very much and we agree that there is a trend that premature stop codon near the end of the protein presented milder symptoms. We have indicated the finding in the results section (Lines 160-162, highlighted in yellow).

8. When categorizing the PEX1 mutation severity, keep in mind that many patients bear compound heterozygous mutations for a severe and milder mutation. This often results in an intermediate phenotype. It can thus be difficult (or impossible) to correlate the mutation with disease severity in these cases. To address this, did you include disease

severity in your tables only for patients with homozygous mutations. This must be discussed and clarified.

Reply: Thank you very much for your suggestion, we are very sorry that we missed the description of consideration of clinical symptom and genetic mutation severity. To assess the genetic severity, we took a previous study with through analysis on mutations in PEX1 protein domains regarding mutation position (doi: 10.3390/ijms20153756) and principles suggested by ACMG regarding mutation charactristics (doi: 10.1038/gim.2015.30). To increase the number (power) in the assessment of genetic-phenotype relation, we include patient with both compound heterozygous and homozygous into analysis, in patients with compound heterozygous, the mutation with milder impact on the PEX1 protein was used to make association with clinical symptoms as assessed by authors of each publication.We have added the description in our revised manuscript. (Lines 140-142, highlighted in yellow)

Other comments:

1. Gene names must always be italicized, and protein names not.

Reply: Thank you very much and we have corrected the errors in the manuscript.

2. Please proofread article to correct the many, very prominent grammatical and spelling errors.

Reply: Thank you very much and your suggestion is in agreement with the other reviewer and editor, we have consulted language editing service with native speakers. Changes was made accordingly throughout the manuscript.

3. Correct the title of Table 3. These are not missense mutations

Reply: Thank you very much for your carefulness, we corrected it in our revised manuscript.

Reviewer B

1. Please refine the format of the abstract. It is a case report, not a clinical trial or other observational studies.

Reply: Thank you very much. We re-formatted our abstract as also suggested by editors.

2. What databases did authors have searched in Ovid ? All? Did authors only search using the method of keywords without subject headings? The present search terms seem could not find enough literature in this area. I would suggest refining the search terms. Besides, add the search strategy in the flowchart too.

Reply: Thank you very much for your suggestion, the genetic cause of the disease was identified in 1997, therefore a searched in all databases in OVID did not result in too many publications to review. We searched keywords without subject headings. The search terms (number of citations) are: #1 pex1.mp. (219); #2 zellweger.mp. (1088); #3 or 1-2 (1227). # limit 3 to 1997-2019 (642). We have added our search strategy in our revised flow chart (Figure 2).

3. Add another paragraph to list both strengths and limitations of this manuscript.

Reply: Thank you very much and we have added a paragraph accordingly (Lines 204-211, highlighted in yellow).