Reviewer's report

Title: Distribution of 5' polymorphisms in PEX1 peroxisome biogenesis disorder patients

Version: 1 Date: 27 June 2011

Reviewer: nancy Braverman

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MS ID : 1695856514536322

The authors report two novel investigations of PEX1:

1. They re-evaluated the two reported polymorphisms in the 5 UTR of PEX1, and found that the transcript start in fibroblasts is 3 'of c.-137. They used in silico analysis to predict that c.-137 is in a promoter region, and not in the 5'UTR as previously predicted by Maxwell et al.

2. They further investigate the hypothesis presented by Maxwell, that the presence of these PMS might influence phenotype in patients with PEX1 mutations. Their findings indicate that these PMs do not predict disease severity in terms of survival over that predicted by the mutation type itself.

This is a noteworthy manuscript providing important findings for PEX1 gene description and phenotype correlations. Overall, this paper would benefit from several editorial comments listed below.

Overall comment: Did the authors check to see if there are other PMs reported in the promoter or 5'UTR that might influence protein amounts and therefore phenotype? Because the authors conclude that 5' PMs do not have to be considered for diagnostic or prognostic purposes. If there are other 5'PMs it would be more accurate to conclude that the two PMs studied do not have to be considered for prognosis.

TITLE: should be changed to reflect the manuscript.
Suggest: Characterization of two common 5' polymorphisms in PEX1 and correlations to survival in PEX1 peroxisome biogenesis disorder patients.
Authors can amend this but the current title does not fully reflect the manuscript.

ABSTRACT

Methods: ‘…were incorporated into a novel genotype-phenotype analysis’ –please provide a brief sentence on the method used to correlate phenotype and genotype.

Results…’we suggest a novel genotype-phenotype analysis for PEX1 patients’ – should be in conclusions. It would be better to state results summary here: that these PM were correlated to genotype in this study, and that they did not correlate to patient survival.
State mutations in abstract
1. 5'PMs were analyzed in relation to the two most common mutations in PEX1, p.Gly843Asp and p.1le700TyrfsX42
2. We show that the first, p.Gly843Asp but not the second, p.1le700TyrfsX42…..

BACKGROUND
Peroxisins are the term used for the proteins encoded by PEX genes, and are NOT the term for the gene itself (Distel etal, J Cell Biol. 1996 Oct;135(1):1-3. A unified nomenclature for peroxisome biogenesis factors).

This reviewer recommends condensing the paragraph on peroxisome biogenesis since this detail is not relevent to this report, and instead include a paragraph about differences in severity in patients with the same PEX1 genotype, and therefore why it is so important to sort out whether these PMs can predict this.

P4 line 9. – I would give credit to the group whose hypothesis you are now investigating and state. Maxwell et al suggested that PMs modulating the PEX1 protein levels……

Correct nomenclature is to use the 3 letter amino acid designation, Gly to Asp, not G to D exchange.

METHODS
Informed consent/ethics approval for this project should be stated.
Collection of patients- 30 patients were chosen- What selection process was used?
Table 5 should be supplementary data.

RESULTS AND DISCUSSION-
Definition of the 5'UTR.
Table 1 can be supplementary, as it is presented unmodified from the TRANSFAC database and does not provide any additional information over the text description.

The last sentence: ‘thus the 5’ UTR previously identified coincides with ORF C7orf64’ is confusing since you have just presented data that shows this is not the real PEX1 UTR region.

5' PMS in a PEX1 patient population
Table 2. Consider changing the 3 columns after patient number, to survival and PMs detected, and then the patient mutations. Patients could be re-ordered according to survival, with the shortest survival at the top and the longest survival at the bottom. This will allow the readers the best chance of evaluating the data you want to present-relationship of survival to PMS and mutation.

You state in the legend that all 4 possible constellations of the 5’PMs were
identified, but there are more than 4 possible constellations and these were not identified: TTGC, CCGC, TTGG, etc. Please explain.

The nomenclature should be consistent- for p.Ile700fs please put in X and amino acid number of premature stop.

A footnote should be added if the homozygous mutations are presumed or proven ie if parents were not tested, a deletion on one allele cannot be ruled out.

**TABLE 3. What does the second column ‘ref’ mean?**
The table shows that all 3 homozygotes have CCGG, but the text states only 2/3.

Genotype-Phenotype correlation.
Should point mutation be changed to missense mutation (MM) since point mutations can also be a nonsense codon. Furthermore, it seems logical that patients with short insertions should not be included in the PM group if they result in frameshift and PTC. Finally, Figure 2 uses a different abbreviation system that is not explained in the text: with deletions D and insertions I. Please be consistent with the text and the figure.

Figure 2. It is difficult to see the differences in the shaded circles representing the difference PMs. It might be better to use different shapes or colors.

**Level of interest:** An article of importance in its field

**Quality of written English:** Acceptable

**Statistical review:** No, the manuscript does not need to be seen by a statistician.

**Declaration of competing interests:**

I declare that I have no competing interests.