Author's response to reviews

Title: Characterization of two common 5' polymorphisms in PEX1 and correlation to survival in PEX1 peroxisome biogenesis disorder patients

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Author's response to reviews: see over
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.

There are no others PM or polymorphisms in the 5’UTR that we are aware of (databases or our own diagnostic sequencing). We find the reviewers’ suggestion helpful and have changed the text (Conclusions/Abstract) accordingly.

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.

We appreciate the suggestion of the reviewer. We have changed the title accordingly.

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

We have expanded the description of the genotype-phenotype analysis and included it in the methods part of the abstract.

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.

We have restructured the results section of the abstract following the reviewers’ suggestion.

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…..

We wanted to keep the abstract short and more accessible for the general reader. Because the manuscript focuses on the 5’ mutations and not on the exonic mutations, we decided to mention the actual two most common mutations for the first time in the background section.

BACKGROUND
Peroxins 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).
We did not want to imply that the term "peroxins" refers to the genes and have corrected this introductory line.

This reviewer recommends condensing the paragraph on peroxisome biogenesis since this detail is not relevant 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.

We have modified the paragraph on peroxisome biogenesis to only briefly sketch the function of PEX1. Furthermore, we have introduced a short introduction about differences in severity in patients with the same PEX1 genotype.

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......

We have included this statement and refer to ref. 19.

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

Throughout the text, we have replaced G843D by p.Gly843Asp.

METHODS
Informed consent/ethics approval for this project should be stated.

We have included the information on informed consent and ethics approval in the methods section.

Collection of patients - 30 patients were chosen - What selection process was used?

Patients were not selected for this study. We included all patients for whom material was available for genetic analysis. We have modified this part.

Table 5 should be supplementary data.

We have moved Table 5 to supplementary data (Now Supplementary table 2)

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.

Table1 was moved to the supplementary data (now Supplementary table 1). The remaining tables were renumbered.

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.
We clarified this point, the respective sentence now runs: “Thus the *PEX1* 5` region, previously defined as UTR [19] coincides with the intergenic regions between *PEX1* and *C7orf64* and would comprise UTRs of both genes.”

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.

To focus the analysis on the 5’ polymorphisms and not introduce a new numbering system for the materials used (to which we also refer by these numbers already used in another manuscript), we kept the order of the table. As survival information is only available for a subset of patients, the table would have been arbitrarily sorted by the availability of these data, and in the end would neither be sorted by patient survival nor by mutations or 5’ polymorphisms.

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.

We thank the reviewer for pointing out that the four constellations described in the paper do not include all the possible constellations. All four constellations can be explained by three alleles: -137C -53G, -137T -53C, and the rare -137C -53C. This is the most parsimonious, but still speculative interpretation.

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

Throughout the manuscript we have adopted the nomenclature p.Ile700TyrfsX42 and we have amended p.Gln1231HisfsX3.

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.

In cases were *PEX1* mutations are indicated as homozygous this was inferred from the absence of signal in the sequencing reaction that would indicate heterozygosity and the absence of a second site mutation. We have now clarified this in the figure legend as suggested by the reviewer.

TABLE 3. What does the second column ‘ref’ mean?

We are now referring to ref. 19 using square brackets.

The table shows that all 3 homozygotes have CCGG, but the text states only 2/3. Genotype-Phenotype correlation.
Thank you for pointing out this mistake. We have corrected the entry in Table 2 (previously table 3) as well as in the text.

**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.

We are now referring to M as missense mutation (in the text and in the figure legend). We have included patients with short insertions that do not result in frameshift and PTC in the M group and we now state this more explicitly in the text. To make the figure easier accessible, we do not use the abbreviations D and I.

**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.**

We replaced the circle outlines by colored circles.

**Reviewer’s report**

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

**Version: 1 Date: 7 June 2011**

**Reviewer:** Ann Moser

**Reviewer’s report:**

**Minor essential revisions:** In the text, Figure 1 is referred to as Figure 1A. There is no reference to table 5 in the text.

We have corrected the reference to Fig. 1 and we refer to Supplementary table 2 (previously Table 5) in the Materials and Methods part.

*Finally, we thank both reviewers for helpful suggestions. Their comments and suggestions have considerably helped improving our manuscript.*