Author's response to reviews

Title: Evaluation of the endoplasmic reticulum-stress response in eIF2B-mutated lymphocytes and lymphoblasts from CACH/VWM patients

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Version: 2 Date: 28 August 2010

Author's response to reviews: see over
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Pr Sabina Alam,  
Senior Scientific Editor BMC Neurology  

Clermont-Ferrand, August 28th, 2010

Object: Response to reviewers, MS 3890058723767528

Dear Editor,

We received the peer review and comments from the referees concerning our manuscript MS 3890058723767528:
Evaluation of the endoplasmic reticulum-stress response in eIF2B-mutated lymphocytes and lymphoblasts from CACH/VWM patients
Laetitia Horzinski, Liraz Kantor, Aurélie Huygue, Raphael Schiffmann, Orna Elroy-Stein, Odile Boespflug-Tanguy, Anne Fogli

We thank you and the experts for these excellent comments. Please find below our point-by-point responses to reviewers. We also join the revised manuscript with all changes made in blue colour.

A) Reviewer #1 (Graham Pavitt)  
- Major Compulsory Revisions:
  1) As suggested by the reviewer, we simplified Figure 2 by presenting the data from patients’ primary lymphocytes (PL) and lymphoblastoid cell lines (EIL) separately and in a manner similar to shown in Figure 1. As the goal of this analysis was to evaluate the possible effect of EBV-transformation of PL on the expression level of four mRNA markers of the ER-stress response, we presented the results as fold-change due to the ER-stress conditions. We first calculated the level of each transcript using the 2^(-ΔΔCt) method. Then for each transcript, the primary Ct data was normalized to beta2M and then the level of each transcript under stress conditions was normalized to its level under normal conditions. This calculation was described in the Material and Methods section and in Figure 2 legend.

  2) Table 1 reports patients’ data and GEF activity measured in lymphoblast. As suggested by the reviewers, we added the reference Horzinski et al., 2009 (Plos One) in the legend of Table 1 and in the text, as these patients have been already described in this recent article.
We also reduced the amount of information presented in this Table 1: we omitted the “sex” and the “age at disease onset” columns.

**- Minor Essential Revisions**
1) At the beginning of the results section page 9, we stated that the effect of ER stress on incorporation of labelled amino acids was measured. But due to restriction of the number of figures, data are not shown in the manuscript. As suggested by the reviewer, we added the mean data ± standard deviation (SD) for controls and patients samples in order to illustrate more quantitatively the absence of difference between patients and controls.

2) Concerning the abbreviations used for primary lymphocyte and lymphoblasts; as suggested by the reviewer, we change them:
- Abbreviation “PL” instead of “LP” for “Primary Lymphocyte”;
- Abbreviation “EIL” instead of “LLB” for “EBV-immortalised lymphoblastoid cell lines” or lymphoblasts.

3) As suggested by the reviewer, we replaced the word “proteic” by “protein” in the text.

4) Table 1: as suggested by the reviewer, we used “DNA” instead of “ADN”, and we placed DNA and protein changes in two different columns.

**B) Reviewer #2 (Mark Peter Ashe)**
**- Major Compulsory Revisions:**
1) See response to point 1 of “Major Compulsory Revisions” from Reviewer #1. We added the reference of our recently published article (Horzinski et al., 2009) in the legend of the Table 1 and in the text of the manuscript.

2) We added the mean data ± standard deviation (SD) for controls and patients samples in order to illustrate more quantitatively the absence of difference between patients and controls with regards to the reduction in labelled amino acid incorporation under stress conditions (page 9). Due to restriction of the number of figures, we add the polysome profile figure to this letter (Figure below) in order to convince the reviewer about the quality of the experiment behind the “data not shown” statement. This figure may be incorporated as part of Figure 1, if the reviewer finds it necessary.

3) A possible explanation for the normal ER stress response in eIF2B-mutated lymphoblasts was added to the discussion (pages 11-12).

**C) Reviewer #3 (Scot R Kimball)**
**- Major Compulsory Revisions:**
1) The antibodies against phosphorylated eIF2alpha and the number of variables the experiment involves do not allow to reproducibly pick subtle changes in eIF2alpha phosphorylation in response to thapsigargin treatment. Although we could not observe major changes, it is quite possible that eIF2B-mutated PL and EIL respond to thapsigargin by reduced eIF2alpha phosphorylation compared to control cells. This may serve as a possible explanation for the lack of difference in ATF4 and CHOP expression in mutated and control cells, as was previously found by van der Kollenburg et al (Ref [15]), who tested the response of EIL to heat-shock. A sentence summarizing this possible explanation was added to the discussion.
2) None of the mutations studied here correspond to mutations in yeast that were previously shown to render the mammalian protein eIF2B resistant to the effects of eIF2α phosphorylation [9,10,14]. We added this information and the possible link between eIF2 phosphorylation and absence of heightened ER-stress response in eIF2B-mutated PL and EIL in the discussion part. Moreover, we added in the discussion that fibroblasts and lymphoblasts from four same patients were used in the Kantor et al study (2005) and in the present one. Therefore, the genotype alone does not explain the absence of enhanced sensitivity to ER stress observed in patients’ lymphoblasts.

3) In Figure 2, the thapsigargin concentration and time of treatment are the same as in Figure 1 so that the results presented in the two figures can be compared. We added this information in the legend of Figure 2.

- Minor Essential Revisions
1) See our reply to point 1/Reviewer #1 and point 2/Reviewer #2, above.

- Discretionary Revisions
1) As suggested by the reviewer, we defined GEF as “guanine nucleotide exchange factor” (page 3)

2) A suggested by the reviewer, we added the corresponding references for the statement that “ATF4-mediated heightened stress response was demonstrated in several eIF2B-mutated models” (page 5, line 13). We also added the review Pavitt and Proud (2009) as a reference ([14]) for this statement.

Quality of written English:
As suggested by the three reviewers, some language corrections have been made in the manuscript and in the Figures legends.

We hope that this revised manuscript will be suitable for publication in the BMC Neurology journal.

Sincerely yours,

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**Supplemental Figure:**

<table>
<thead>
<tr>
<th>No stress</th>
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**Polysomal profiles of control and eIF2B-mutated lymphoblasts.** $1.10^7$ cells from control and eIF2B-mutated patients were treated with 2 µg/ml thapsigargin for 30 minutes followed by analysis of the polysomal profiles on 0-50% sucrose gradients. Free ribosomal subunits (40S and 60S), monosomes (80S) and heavy polysomes are indicated.