Author's response to reviews

Title: AlphaA-Crystallin R49Cneo Mutation Influences the Architecture of Lens Fiber Cell Membranes and Causes Posterior and Nuclear Cataracts in Mice

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Version: 4 Date: 25 June 2009

Author's response to reviews: see over
June 25, 2009

MS: 1736373939244349

AlphaA-Crystallin R49Cneo Mutation Influences the Architecture of Lens Fiber Cell Membranes and Causes Posterior and Nuclear Cataracts in Mice
Usha P Andley

Dear Dr. Graham,

Thank you very much for your e-mail of June 24, 2009 regarding our manuscript. I have addressed the remaining concerns of Dr. John West’s review in the revised manuscript. My point-by-point response and the revisions made are summarized below:

1. Comment: “In the new text on page 8 (line 1). The author refers to a method by Pierce; the appropriate reference should be included.”

Response: On Page 8, line 1: The protein concentration was determined by bicinchoninic acid (BCA) Protein Assay according to the manufacturer’s instructions (Thermo Scientific-Pierce Chemical Co, Rockford IL). I have added this to the text of the revised manuscript (Page 8, line 1-2).

2. Comment: “Rather than including one example of an assay result the author should summarise the quantitative results of several experiments and indicate which results are significantly different.”

Response: The results are representative of 6 lenses per genotype. The analysis was performed twice with almost the same results. I have added this sentence to the text of the revised manuscript (Page 9, line 3-5).

3. Comment: “Figure 2...We are told that the aim is to compare WT/R49Cneo and R49Cneo/R49Cneo lenses to previously published results for WT/R49C and R49C/R49C. In the Discussion WT/WTneo and WTneo/WTneo lenses are also compared to wild-type (see point 3.2 below). Other genotypes should be included in the chromatography analysis to make the relevant comparisons.”

Response: We have already published the results for WT/R49C and R49C/R49C lenses in our previous publication (Biochemistry, 2008; volume 47, pages 9697-9706). The chromatography instrument and detection systems used for the published chromatographic analysis was different from the one used in the current manuscript. Therefore, I have not included that analysis in Figure 2 of the present manuscript. In response to the Reviewer, I have included the following sentence in the text:
As compared with WT/R49C lenses, α-crystallin decreased more in the WT/R49Cneo lenses (Page 10, line 4-5).

4. Comment: “3.2. The new sentence ‘In the present work, no significant defects were found in WT/WTneo and WTneo/WTneo lenses, assuming that introduction of the neor gene does cause a reduction in gene expression.’ (Page 15, paragraph 2) does not make sense and should be re-written. Ideally the author should determine whether alpha-crystallin levels in WT/WTneo and WTneo/WTneo lenses are significantly less than in wild-type lenses (see point 3.1 above) and then relate this conclusion to the phenotypic observation that WT/WTneo and WTneo/WTneo lenses show no significant defects compared to wild-type lenses.”

Response: In response to the comment, I have modified this sentence on page 15, paragraph 2. It should be pointed out that small difference, if they existed, in α-crystallin levels of WT, WT/WTneo and WTneo/WTneo are not expected to lead to phenotypic differences between these three types of wild type lenses. It is the presence of R49Cneo that leads to the phenotypic changes in WT/R49Cneo lenses. This is because the R49C mutant protein may affect the lens in a negative, toxic manner.

4. Comment: “Discretionary Revision 3.3 The legend to Fig 2 should explain the units used (mAU).”

Response: mAU represents milli absorbance units at 280 nm for the UV detector. I have added this to Figure 2 legend in the revised manuscript.

I believe that I have made all the corrections requested by the Reviewer, and hope that you will now consider this manuscript suitable for publication in *BMC Ophthalmology*.

Thank you once again for your excellent Editorial help.

Best regards,

Usha

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