Reviewer's report

Title: AlphaA-Crystallin R49C Mutation Influences the Architecture of Lens Fiber Cell Membranes and Causes Posterior and Nuclear Cataracts in Mice

Version: 1 Date: 29 January 2009

Reviewer: John West

Reviewer's report:

Referees Comments to Author

This manuscript describes an interesting set of observations but needs some reorganisation. The Introduction and Discussion are good but the aims are not properly specified and the description of the results lacks details. Some text that should be in the Results appears in the figure legends and methods section. Also it may be better to combine some of the figures.

Major Compulsory Revisions

(which the author must respond to before a decision on publication can be reached)

(1) A clear statement should be included at the end of the Introduction to explain that the aim of the study was to characterise the lens abnormalities in WT/R49Cneo and R49Cneo/R49Cneo mice and compare them to previous reports of WT/R49C and R49C/R49C lenses (refs 44 and 55). Currently this only becomes really clear once the reader reaches the Discussion.

(2) The results are mostly presented as a series of illustrations without indicating how representative they are. The number of mice of each genotype that were examined at each age should be stated with an indication of how variable the results are.

(3) The methods used to determine the percentage water insoluble alphaA-crystallin protein (Fig 3) should be included in the Materials and Methods. The two panels of Fig 3 should be labelled 'A' and 'B' and should be described more fully in the legend. In the first panel of Fig 3, the 3 lanes differ in genotype. The age and cataract severity should be given in the legend. The second panel shows estimates of percentage of insoluble protein for lens with cataracts severities 1, 2 and 3. The histogram should show error bars and the legend should explain how many samples there were per severity class and what genotypes and ages they were.

(4) Fig 7C legend should explain what the cell numbers mean (e.g. cell numbers per section or field of view or lens?) Should genotypes WT/R49C and R49C/R49C be WT/R49Cneo and R49Cneo/R49Cneo in 7C?

(5) P8: In the Discussion it seems to be assumed that R49Cneo causes reduced
expression of alphaA-crystallin (e.g. ‘These lower levels of R49C alphaA-crystallin evidently have given us a novel way to examine the effect of lower expression levels of the mutant R49C alphaA-crystallin protein on lens opacity and histology.’). Is there any direct evidence for this or it is speculation?

(6) Fig 4 should show all three genotypes at both 3 days and 3 months (so a 3 day homozygous R49Cneo/ R49Cneo and a 3month heterozygous WT/ R49Cneo should be included.

Minor Essential Revisions
(such as missing labels on figures, or the wrong use of a term, which the author can be trusted to correct)

(7) Figure legends: It would better if the figure legends were used to explain fully what the figure shows and other comments moved to the text of the results section. For example, in Fig 3 legend the sentence ‘The increased severity of cataract after deletion of the neo cassette is putatively caused by an increase in R49C-alpha-A crystallin expression.’ would be better in the main text of the Results. Similarly some of the commentary in Figs 6 and 7 would be better in the text.

(8) Page 5/last paragraph: The Methods section should not include results such as ‘Changes appear by 3 weeks in heterozygous and homozygous lenses’ etc. This type of information should be in the Results.

(9) Fig 1: The legend should clarify whether the asterisk above exon 1 indicates the mutation and should indicate that the numbered blue rectangles are exons, the filled triangles are loxP sites and ‘X’ is the Xhol site. It might also be worth modifying the diagram slightly because the lines illustrating recombination between the construct and the wild type allele could be misinterpreted as unequal crossing over. On the right, one line goes from intron 1 to intron 3 whereas the other one goes from intron 1 to intron 2.

(10) The age of the mice shown in Figs 2B and 2C should be given in the legend.

Discretionary Revisions
(which are recommendations for improvement but which the author can choose to ignore)

(11) The title is a bit misleading because the manuscript is about the R49Cneo mutation not R49C.

(12) p3/para 1/line 12: Consider whether ‘lens membranes’ would be better as ‘lens cell membranes’ (e.g. to avoid confusion with capsule)?

(13) P5/para2/3: ‘Cre-recombinase sites’ should be changed to ‘loxP sites’ which is more accurate and consistent with the Results (line 4)

(14) p5/2/5: The mouse strain name should probably be ‘129/SvJ’ rather than ‘129SvJ’
(15) p5/2/12 and 13: The mouse strain name should be given in full (‘C57BL/6’ rather than ‘C57’ or ‘C57BL6’)

(16) It may be better to combine some of the figures.

**Level of interest:** An article whose findings are important to those with closely related research interests

**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