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:** 7 February 2009

**Reviewer:** Jochen Graw

**Reviewer's report:**

Dr. Andley describes in her actual manuscript some additional morphological details of the already published R49C transgenic Cryaa-mutants; moreover, she adds a few data on a hypomorphohic allele lacking the neo-cassette. The actual manuscript is the third in a line of two already published papers on the R49Cneo transgenic line.

The main problem of the actual manuscript is the impression that a new transgenic line is described. This impression is formed by sentences in the Abstract like "we have produced a new mouse cataract model ..." (lines 4-7), or by the last paragraph in the Introduction. The reader assumes that a new transgenic mouse line has been described in the actual manuscript according to previously reported transgenic lines. Only if one is looking in detail for the references 44 and 55, it becomes obvious that these papers deal with the same mutants. Particularly, the Introduction does not mention the previous findings as starting point for the experiments reported in the new manuscript.

Moreover, the Material-and-Method section of the new manuscript gives a very detailed repetition of the entire procedure generating the knock-in mice. However, it has been already described in the same detailed manner in the previous paper by Xi et al., 2008; it includes the names of the lines produced and tested (KI3 and KI4) and the primer sequences for genotyping. To avoid misunderstanding, the entire paragraph ("generation of knock-in mice") has to be skipped in a revised version.

Also some of the figures given in the new manuscript are similar to those of the previous papers: Fig. 1 is more or less identical to Fig. 1a of the paper by Xi et al., 2008, and the lens phenotypes given in Fig 2a-f give the same information for 2 months and 4 months as previously reported for 5 and 16 weeks (Xi et al., 2008, Fig. 3a-f). However, the already published figures are slit lamp pictures with densitograms giving much more information than the pictures in the new manuscript.

Figure 3 of the new manuscript gives just an acrylamide gel for water-soluble lens extracts (there is no corresponding method given - just a reference to Immuno-bLOTS, but it seems to be a Coomassie-staining). The immunoreactivity, which is summarized on the right side of this figure, was already reported in much more detail in both previous papers (Xi et al., 2008; Andley et al., 2008 - references 44 and 55). As a minor point in this context, the legend of the figure is not helpful for understanding (amount of protein? age and number of
animals/lenses? single or pooled lens extract? etc); in the right part, the number of extracts tested is missing as well as error bars (either for SD or SEM).

Moreover, the immunofluorescence analysis of the R49C transgenic mice using a MIP antibody (Fig 8 of the new manuscript) was described also previously in more detail in the paper by Xi et al., 2008 (Fig. 9).

The new aspects of the manuscript are in the figures 4-7 and 9 giving a detailed morphological analysis including electron microscopy; also Fig. 2g, h gives new information for the mutant line lacking the neo-cassette and giving rise to a hypomorphic allele.

If Dr. Andley wants to submit a revised version, she should make clear in the abstract and more detailed in the Introduction that the new manuscript continues the characterization of already existing transgenic lines. In the Introduction, the previous findings should be shortly summerized and the aim of the new study should be emphasized. The result section should deal with new data only.

If the results of the transgenic mice without the neo-cassette will be demonstrated, the promoter of the Cre gene should be mentioned to understand in which tissues the neo-cassette will be lost. Moreover, instead of speculations about the reduced number of transcripts in some of the transgenic lines (p8, para 1 and 2), one can make the corresponding experiment and determine their relative concentration using real-time PCR.

Minor points:

The genetic symbol Cryaa (encoding aA-crystallin; in italics) should be used throughout.

The revised version should be written with 1.5 line spacing; the Introduction and Discussion should be shortened to 1.5 and 2 pages, resp.

Finally, it is surprising that an experimental paper is written by one author only.

In conclusion, the manuscript needs major revision and re-review.

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.