Retina Australia 2016 Project Grant Report

TITLE: Cone photoreceptor development and cell death mechanisms during retinal degeneration in mouse models of Achromatopsia

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BACKGROUND

Achromatopsia (ACHM), also referred to as rod monochromacy, is a devastating early-onset disease estimated to affect 1:30,000-50,000 people worldwide. Clinical symptoms start at birth/early infancy and include not only completely absent colour discrimination with no recordable electroretinogram (ERG) cone function, but also congenital pendular nystagmus, poor visual acuity, severe photophobia and hemeralopia. So far, mutations in five genes have been shown to cause achromatopsia, and for four of these genes, mouse models that replicate the disease exist. In all cases, the genes encode for key components of the visual phototransduction cascade of cone-specific genes. However, since in the mouse retina cone photoreceptors represent only 3% of the total number of photoreceptors, it has been historically difficult to study these cells in isolation. One of the main aims of this project was therefore to cross ACHM mouse models with a transgenic mouse where the cone cells express a fluorescent protein specifically in cone cells to facilitate the study of these cells.

Using these newly created mouse lines, the overall objective of this project was to define the factors that trigger cone cell death and which apoptotic pathway are being utilised in ACHM, and investigate for the first time if there is a causal link between impaired cone migration during development and cone loss by apoptosis. Additionally, these models will also help us understand the effects these mutations can have on cone cell migration, maturation and survival.

PROJECT PROGRESS

Animal lines and colonies establishment: Some delays were experienced in this process due to the fact that all animal lines were initially going to be coming from the Institute of Ophthalmology at University College London (UCL, UK) but since the lines did not pass the health clearing tests for import into the UWA Biomedical Research Facility (BRF), alternatives had to be sought for import of the lines. This was done by sourcing the lines directly from their original depository. Two ACHM lines ($Pde6c^{-/-}$ and $Gnat2^{-/-}$) were available from the Jackson lab (JAX) while a third ACHM line ($Cnga3^{-/-}$) was available from the KOMP UC Davies depository and the GFP cone reporter line (Chnrb4.GFP) was purchased from the MMRRC. All individual lines were imported and cleared from quarantine in March-April 2016 and have now been established as successful colonies.

The reporter GFP fluorescent line to be used in this study (Chrnb4.GFP line) was in a FVB background while all other mutant lines are on a C57bl/6 background. To allow for comparable genetic studies, the Chrnb4.GFP line and all crosses had to be backcrossed into the C57bl/6 background. Colonies for the three ACHM lines crossed with the reporter line have now been established and Figure 1 confirms the specificity of the green fluorescent protein (GFP) expressed in the reporter line in cone cells only.

We were also able to establish a protocol for isolation of a pure cone photoreceptor populations by FACs. Retinas from the wt line were used to test out the retinal cell dissociation protocol and undergo

FAC sorting to quantify the numbers of live GFP+ cone cells that could be isolated for use in genetic studies of cell death mechanisms. Following a papain dissociation if retinal tissue, a live cell population was FACs and we were able to detect around 11% of GFP+ cone cells (Figure 1).

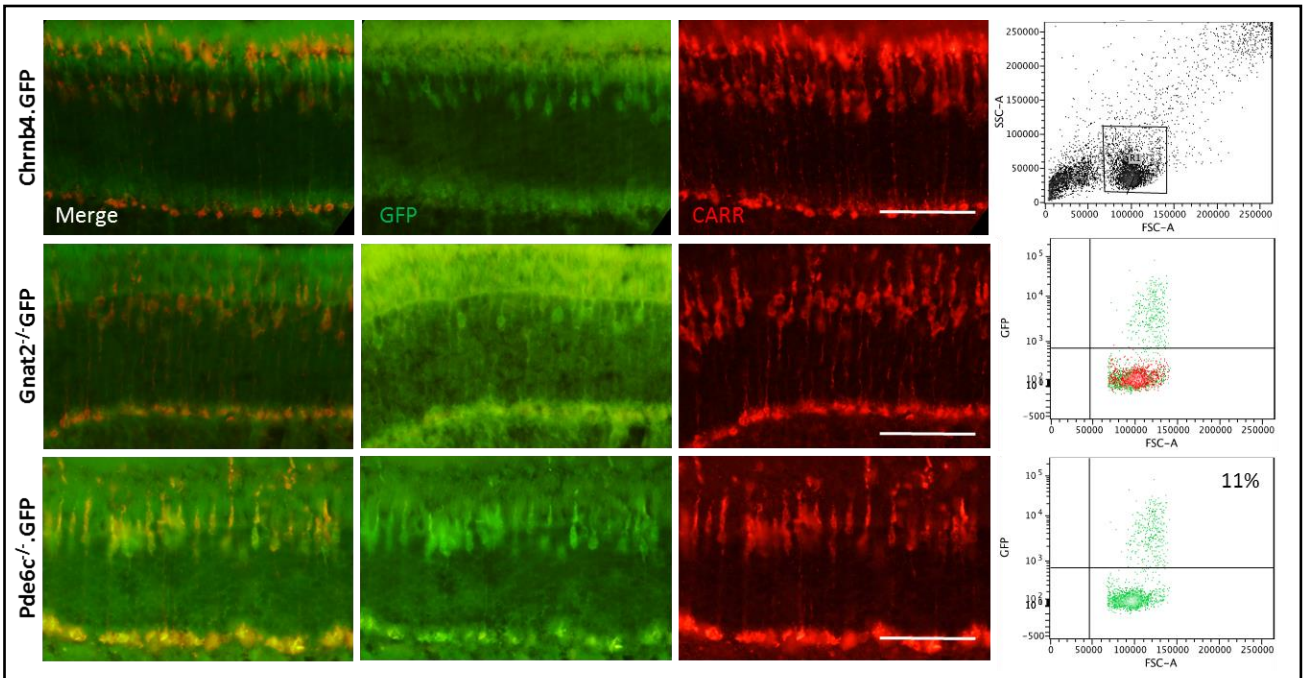

Figure 1. Retinal sections of GFP expressing cone photoreceptors (green) co-labelled with a cone-specific marker cone arrestin (CARR – red). Scale bar = 500µm. Left panels: FACS analysis of dissociated cells from the Chrnb4.GFP retina gated for GFP+ cells. Bottom graph shows the % of GFP+ cones from the total of viable (alive) cells.

***Cone photoreceptor cell death*:** One of the objectives of this project was to define the peak time point of cone cell death in these models and establish the presence of the link between apoptosis of cone cells and their position within the outer nuclear layer (ONL) of the retina. A protocol for apoptosis detection (TUNEL) has been established using the ApopTag® Red In Situ Apoptosis Detection Kit (Millipore). Comparison of TUNEL positive cells through development between wildtype (WT) and Cnga3$^{-/-}$ retinas shows that a normal pruning process occurs during development (at P8-P11) but that at P24 this is finished in the WT retina whereas the Cnga3-deficient retinas sees an increase in TUNEL positive cone cells (Figure 2). We also found that quantification of TUNEL positive cones cells in WT and all three mutant models at P24 found a significantly higher number of cell located at the wrong part of the outer nuclear layer (mislocalised).

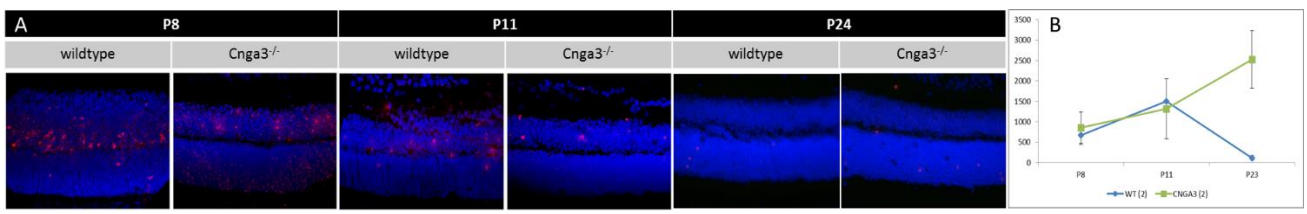

Figure 2. (A) Retinal sections of Cnga3-deficient model showing TUNEL positive cells (red). (B) Quantification of TUNEL positive cells at different developmental time points between Cnga3-/- and wildtype.

Cone migrations defects in ACHM: we were also able to show that in all three models, knocking out expression of proteins of the phototransduction cascade seems to result in an impaired/delayed migration of cone photoreceptors in the outer nuclear layer as seen by several misplaced cone nuclei at P12 in the three ACHM models compared to wildtype (Figure 3).

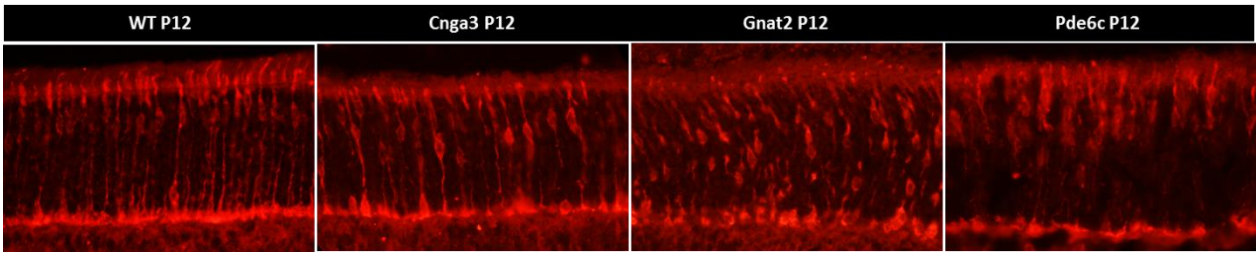

Figure 3. Retinal sections showing cone arrestin positive cone cell nuclei (red) at 12 days of age (P12) in wildtype and achromatopsia retinas. Note misplaced cone nuclei in *Cnga3*, *Gnat2* and *Pde6c* models.

CONCLUSIONS

The Retina Australia Grant was fundamental in enabling us the import and establishment of these novel mouse model lines to study specific cone degeneration mechanisms. We are well into the characterisation of these models and should shortly be able to start molecular and genetic analysis of cone death pathways of isolated pure cone cell populations. The establishment of these novel lines has already provided the opportunity for national and international collaborations and part of ARC and NHMRC grant application currently under consideration.