

Summary of project funded by Retina Australia 2010.

Title: *Histamine in the development and maintenance of the retina*

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Background:

We made the interesting observation that mouse mutants, which cannot produce histamine (HDC-KO), show an aberrant phenotype in the outer retina reminiscent of photoreceptor diseases as retinitis pigmentosa and Leber's Congenital Amaurosis. From this we hypothesised a role for histamine in the development and maintenance of the retina, and in particular photoreceptors. We proposed to study the role of histamine in photoreceptor development and the consequences of histamine deficiency for the photoreceptors.

Experiments and Results:

Our experiments characterized the nature and time course of disruption in retinæ of HDC-KO mice. We established that first signs of disruptions (displaced photoreceptors) occurred early post-natally (postnatal day 7). Thereafter the disruptions became more severe, and in adulthood (2 months) large retinal regions lose their photoreceptors.

Most interesting we established that the Outer Limiting Membrane (OLM) was disrupted in the HDC-KO mice. The OLM is an important structure in the outer retina that maintains the proper orientation of the photoreceptors and therefore the close relationship of photoreceptors with the underlying Retinal Pigment Epithelium, which is crucial for their survival. The OLM contains specialized adherens junctions between photoreceptors and adjacent Müller cells, which contain multiprotein complexes (containing ZO-1, β -catenin, crumbs). Mutations in genes encoding these proteins (e.g. *Crb1*) are associated with photoreceptor death in Leber's Congenital Amaurosis in both transgenic mice and humans.

We found histamine to be expressed in the photoreceptor layer and histamine receptors expressed on Müller cells (Greferath et al., 2009). Based on our data we propose that histamine is an important regulator of OLM adherens junctional protein expression by Müller cells, and has a key role in maintaining photoreceptor polarity and viability.

We are in the process of submitting a manuscript to *Human Molecular Genetics* and have submitted an application for funding in 2012 from NHMRC.

What do our results mean for retinal degeneration:

Much of what we have learned about photoreceptor death has come from retinal degenerations caused by genetic mutations in rod associated proteins (e.g., rhodopsin) that cause death of rod photoreceptors followed by cone photoreceptors. Inherited retinal degenerations, however, represent a *family* of disorders, with a number of them caused by mutations affecting other cell types (Fletcher et al 2011). Indeed, recently, a number of retinal degenerations have been attributed mutations in genes that regulate the Outer Limiting Membrane (OLM), an important region that maintains the close relationship of photoreceptors with the underlying Retinal Pigment Epithelium. 10-15% of cases of Leber Congenital Amaurosis, the most severe form of inherited retinal degeneration, and also approximately 4% of cases of

autosomal recessive Retinitis Pigmentosa (eg., RP12) are caused by a gene that disrupts the OLM. In addition, an impaired OLM has been correlated with reduced visual acuity in those with Age-Related Macular Degeneration. However, very little is known about neither the pathogenesis of these forms of retinal degenerations, nor mechanism(s) by which defects in the Outer Limiting Membrane lead to photoreceptor degeneration. Our novel findings in the HDC^{-/-} mouse suggest that histamine is important for regulating Müller cell expression of adherens junctional proteins. The information gained here will provide the first indication of the pathogenesis of these unusual forms of retinal degeneration and provide the first step towards development of possible treatments.