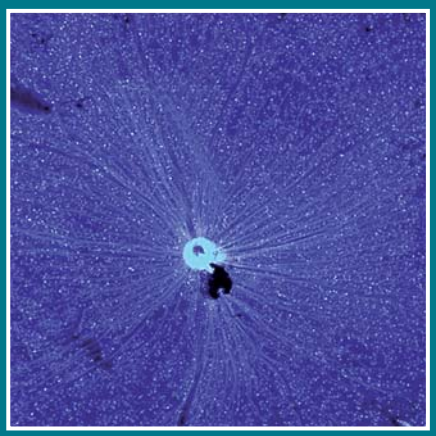
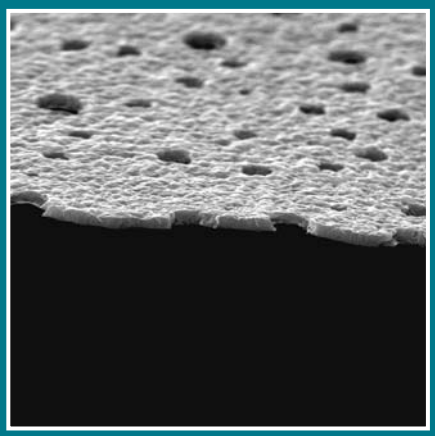
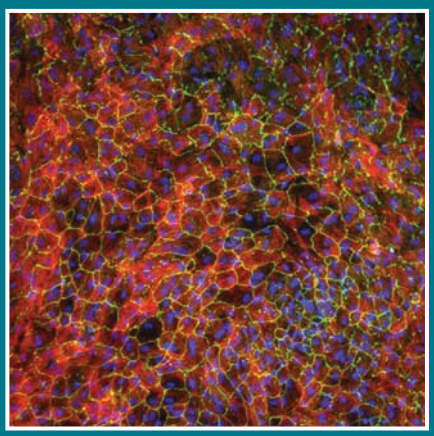
Annual Report 2010

ORIA







The Ophthalmic Research Institute of Australia

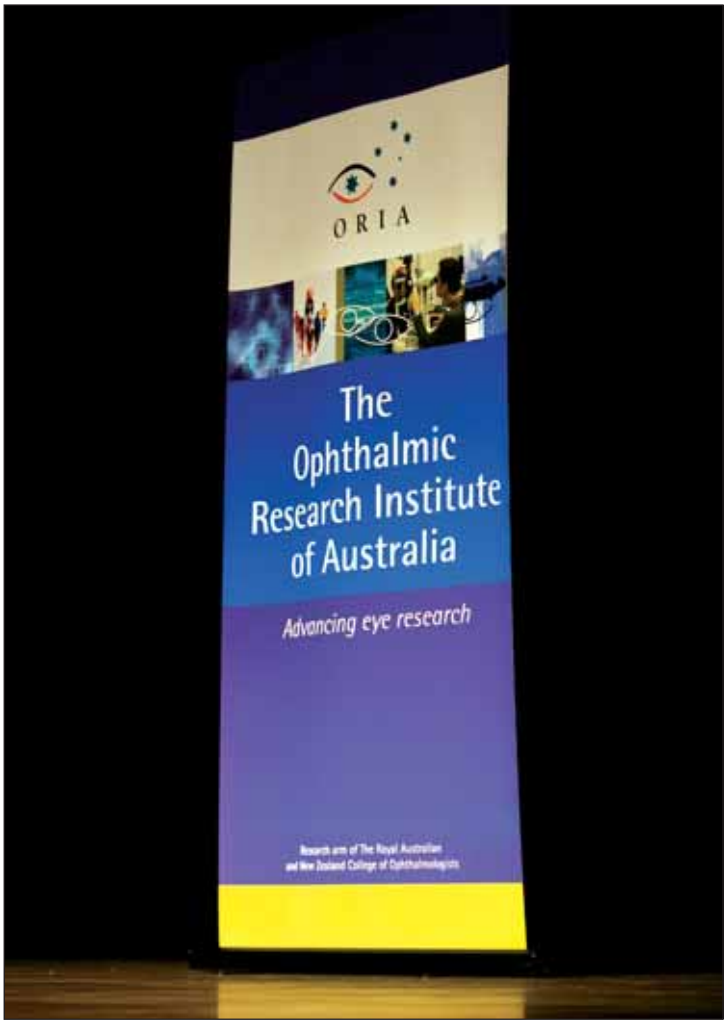
RESEARCH REPORTS AND FINANCIAL STATEMENTS

The Ophthalmic Research Institute of Australia

94-98 Chalmers Street, Surry Hills NSW 2010
Tel: (02) 8394 5218 Fax: (02) 9690 1321
Email: asnape@ranzco.edu Web: www.oria.org.au

Notice of Meeting

The Annual Report will be presented at the Fifty Eighth Annual General Meeting to be held in Adelaide, South Australia on Sunday 21 November 2010 at 8.00 am.



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HONORARY TREASURER	A/Prof Robert Casson 94-98 Chalmers Street, Surry Hills 2010
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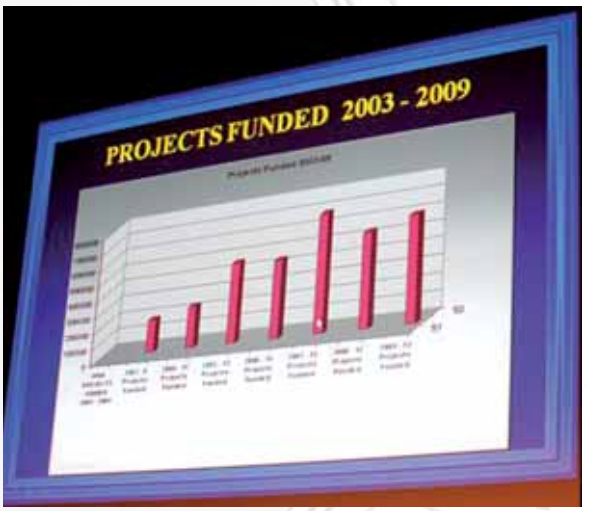
EXECUTIVE OFFICER	Ms Anne Dunn Shape, BA, GradCert Ethics&LegalStuds

Chairman's report

The Ophthalmic Research Institute of Australia is the College's research arm, and aims to "Advance Eye Research". The ORIA's activities are co-ordinated and managed by the sixteen member Board of the ORIA and Executive Officer, Anne Dunn Snape.

Using the income from its investments and donor organisations, the ORIA continued to contribute to funding for research projects throughout Australia. During the year the ORIA's Research Advisory Committee considered 36 applications for project funding from Australian researchers, a significant increase from 19 assessed in 2004. It also assessed three New Zealand applications for funding on behalf of the Save Sight Society of New Zealand. The NZ Branch is represented on the committee via its Save Sight Society.

The ORIA's Research Advisory Committee is composed of leading research scientists and ophthalmologists from Australia and New Zealand. All applications are independently peer reviewed which forms the basis for discussion and recommendation of funding by the Committee. The recommendations of



An ORIA presentation at its AGM 2009

the Committee are put forward to the Board of the ORIA who then indicate what funds are available for the forthcoming calendar year. This year $596,600 was distributed to fund 13 one-year projects. The College donated $40,000 and the RANZCO Eye Foundation a further $75,000. Glaucoma Australia Inc. donated $45,790 to co-support a project. The ORIA continued funding a New Investigator category in an endeavour to encourage up-and-coming researchers; three grants were awarded this year.

Significant projects to receive funding were:

ORIA/RANZCO Grant
Prof K A Williams and Prof D Coster
Improved Screening for eye disease in premature babies
$50,000

ORIA/Esme Anderson Grant
Dr S Wickremasinghe and Prof R Guymer
Does genotype influence response to anti VEGF therapy? A prospective study
$43,550

ORIA/R & L Lowe Grant
Prof M Gillies
A transgenic model for selective ablation of Müller cells
$49,960

ORIA/Renensson Bequest Grant
A/Prof J Craig and Dr K Laurie
Identification of a novel gene for Nanophthalmos in a large Australian pedigree
$49,200

Details of all other grants awarded can be found on the ORIA website www.oria.org.au and for New Zealand at www.safesightsociety.org.nz.

The ORIA, as a significant funding body for eye research in Australia, has conducted two audits over the previous three years in an attempt to measure outcomes from the research it has funded. In 2002 the $250,00 distributed in grants provided the basis for more than $2.5million in federal funding. In 2003, a similar amount funded by the ORIA for eight projects, resulted in $3.5million of additional funding. All of the successful researchers were still involved actively in research and this funding provided a track record and credibility that further enhanced their reputations and so allowed them to gain these larger grants. As well, the audits have confirmed that the ORIA continues to deliver best practice with its own internal systems.

Part of the ORIA's strategic plan is to promote its role to College Fellows. At an ORIA symposium at the Annual Scientific Congress of the College in Brisbane, Queensland, the Institute presented progress on projects funded in 2007 and 2008 to Prof David Mackey, Dr Paul Baird, Prof Keryn Williams and Dr Kathryn Williams. The theme of this symposium was genetics.

The ORIA has continued its support of the Australasian Visual Sciences Meeting. It was decided not to hold a separate meeting this year, but to attempt to reinvigorate interest by holding it concurrently with RANZCO. A meeting will be held on Sunday 21st November at RANZCO, Adelaide, South Australia and all fellows are encouraged to attend.

The ORIA also continues its annual support of the Ringland Anderson Chair of Ophthalmology in Victoria.

During the year, the ORIA saw some changes to its Board with Dr Ehud Zamir and Dr Salmaan Qureshi retiring. New members to the ORIA Board are Dr John Males and RANZCO nominee, Dr Wilson Heriot.

***Mark Daniell**, Chairman, ORIA*

ORIA grants awarded in 2010

ORIA/RANZCO GRANT
PROF K A WILLIAMS AND PROF D COSTER
Improved Screening for eye disease in premature babies
$50,000

ORIA/ESME ANDERSON GRANT
DR S WICKREMASINGHE AND PROF R GUYMER
Does genotype influence response to anti VEGF therapy? A prospective study
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PROF M GILLIES
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$49,960

ORIA/RENENSSON BEQUEST GRANT
A/PROF J CRAIG AND DR K LAURIE
Identification of a novel gene for Nanophthalmos in a large Australian pedigree
$49,200

ORIA GRANT
DR K BURDON AND DR R MILLS
Identifying genes for keratoconus from a genome wide association
$49,650

ORIA GRANT
DR S WATSON, A/PROF L FOSTER AND DR M SARRIS
Development of Antimicrobial Sutureless Technology for eye surgery
$49,950

ORIA/RANZCO EYE FOUNDATION GRANT
DR P SANFILIPPO AND PROF D MACKEY
The heritability of optic disc shape
$28,700

ORIA GRANT
DR L ROBMAN
Age-specific genetic profiles for age-related macular degeneration
$46,600

ORIA GRANT
PROF P McCLUSKEY, DR M MADIGAN, DR R M CONWAY AND PROF N RAO
Do patients with VKHD develop specific immune responses against uveal melanocytes?
$49,900

ORIA NEW INVESTIGATOR/RANZCO EYE FOUNDATION GRANT
DR L LIM
The role of inflammatory biomarkers in ranibizumab resistance
$48,100

ORIA NEW INVESTIGATOR/GLAUCOMA AUSTRALIA INC GRANT
MS F O'HARE
Characteristics of auditory function in individuals with glaucoma
$45,791

ORIA NEW INVESTIGATOR GRANT
DR M SCHACHE
Do changes in DNA structure cause shortsightedness?
$49,905

ORIA NEW INVESTIGATOR GRANT
DR J S GILHOTRA AND DR M DHANAPALA
A study of treatment for retinal vein occlusion with Avastin
$35,435

TOTAL AWARDED $596,741

The ORIA's Research Advisory Committee at the Chalmers Street headquarters

THANKS

With many thanks to:

Glaucoma Australia Inc

In Memoriam Neil and Molly Lamerton

RANZCO

RANZCO Eye Foundation

Progress reports on research supported by ORIA Institute grants 2009

Improving outcomes for corneal transplants 2008–9

A/Prof KA Williams, Dr HM Brereton and Prof DJ Coster

ORIA/RANZCO Grant

Overview and aims

Corneal transplant surgery can restore vision to many people who are visually impaired. However, a significant proportion of such transplants fail because the recipient recognizes the transplant as being foreign and rejection ensues. Our goal is to reduce the impact of rejection by transferring genes to the donor cornea *ex vivo*, prior to surgery. We have prolonged corneal graft survival significantly in an outbred experimental animal model by such an approach using adenoviral vectors, but have been unable to produce long-term expression of the therapeutic gene so that the grafts ultimately fail. We are now exploring a lentiviral vector as a means of long-term gene transfer to the cornea. Our specific aims are:

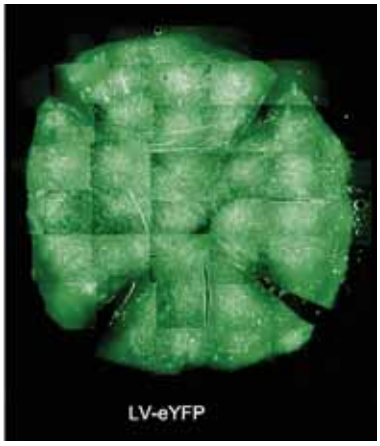
(i) to construct and characterise lentiviral vectors carrying transgenes controlled by a constitutive promoter, or by a glucocorticosteroid-inducible element;

(ii) to construct and test multicistronic lentiviral vectors containing the novel 2A sequence, to allow expression of multiple transgenes in the cornea;

(iii) to test these vectors for their ability to prolong rat and ovine corneal allograft survival.

Background and progress

Our gene therapy vectors, designed by Professor Donald Anson, are recombinant HIV-1-based lentiviruses (LV) that are pseudotyped with vesicular stomatitis virus glycoprotein G, and that are non-replicative and self-inactivating. The basic vector contains the strong viral promoter, SV40. We assessed transgene expression in corneal endothelium following transduction with lentiviral vectors containing a number of other promoters. Vectors containing the phosphoglycerate kinase promoter, the elongation factor-1 alpha promoter, or the myeloproliferative sarcoma virus promoter were not significantly more effective than the basic vector in driving transgene expression. Two lentiviral vectors containing the SV40 promoter and either the ovine or the rat interleukin 10 (IL10) transgene were then constructed. Lentivirusmediated expression in transduced ovine and human corneal endothelium was assessed by fluorescence microscopy, real-time quantitative reverse transcription PCR (qRT-PCR) and ELISA, following alterations of transduction period duration (2–24 hours) and vector dose, as well as in the presence or absence of polybrene. A 24 hour transduction of ovine corneal endothelium with the lentiviral vector encoding IL10 resulted in expression levels which were increasing after 15 days of organ culture but logarithmically lower than those achieved by adenovirus. Shortening the lentiviral transduction period to two hours led to a reduction in expression, but the addition of polybrene (40 μg/ml) to the transduction mixture restored expression to levels comparable to those attained after a 24 hour transduction period, although at the cost of associated toxicity for corneal epithelium. Ovine corneas were transduced *ex vivo* with a LV-SV40-interleukin 10 (IL10) construct and transplanted orthotopically to the eyes of recipient sheep. Corneal allograft survival was prolonged by a median of seven days in the LV-SV40-IL10-treated recipients, compared with the control group ($p=0.026$). Thus although the lentiviral vector showed some promise for corneal gene therapy, we considered further vector modification to increase transgene expression, and the use of multigenic vectors was warranted, to attempt to further prolong corneal allograft survival.



Human cornea, en face, transduced with lentiviral vector carrying the eYFP transgene.

Aim 1

We first compared gene expression controlled by the SV40 and the cytomegalovirus (CMV) promoters. The CMV promoter produced faster, stronger transgene expression in ovine 2 cornea than did the SV40 promoter, and is probably the construct of choice. We have also assessed the efficacy of a glucocorticosteroid-inducible promoter in controlling transgene expression following lentivirus-mediated gene transfer to ovine and human corneas. A glucocorticosteroid response element (GRE5) was cloned into a lentiviral vector (LV-GRE-IL10) encoding the model transgene interleukin 10. Transgene expression by LV-GRE-IL10-transduced A549 cells, ovine corneas, and human corneas cultured with or without dexamethasone, was quantified by an IL10-specific enzyme-linked immunosorbent assay. IL10 levels were 30-40-fold higher in supernatants from LV-GRE-IL10-transduced A549 cells cultured with dexamethasone than in controls.

Dexamethasone withdrawal resulted in restoration of baseline IL10 levels. Supernatants from LVGRE-IL10-transduced ovine and human corneas cultured in dexamethasone contained 9–10 times more IL10 than supernatants from transduced corneas cultured without dexamethasone. We conclude that the GRE5 promoter in a lentiviral vector can drive rapid, sustained and inducible transgene expression in both ovine and human corneas in the presence of dexamethasone. A steroidinducible promoter may be particularly useful for controlling transgene expression in gene-modified donor corneal allografts, given that topical glucocorticosteroids are administered to virtually every corneal transplant recipient.

Aim 2

Given the complexity and redundancy of the immune response to a foreign graft, it has always been apparent to us that the use of a cocktail of gene therapy vectors, each carrying cDNA for a different therapeutic transgene, or the use of a multicistronic vector carrying multiple transgenes, might be necessary to achieve the maximum therapeutic effect. Lentiviral vectors typically have a reasonably-sized expression cassette and in theory, can carry more than one transgene. Incorporation of a so-called 2A sequence from a Picornavirus such as foot-and-mouth disease virus (FMDV) into the expression cassette of a viral vector, between multiple transgene sequences, results in production of a polyprotein expressed from a single open-reading frame. The polyprotein then self-cleaves at a post-translational level into individual proteins at the 2A motifs.

Using this system, efficient production of multiple, functional transgenic proteins in a single cell has been reported. We have now constructed vectors containing two transgenes separated by the 2A sequence. Transgene expression at the mRNA and protein levels in cell lines and in rat corneas *in vitro* was quantified by qRT-PCR and by flow cytometry, respectively. Transgenes are expressed in the appropriate cellular compartment, but the second transgene in the string is expressed to a lower level than the first, irrespective of the order of genes in the string.

Aim 3

A modest but significant prolongation of corneal allograft survival in both the rat and the sheep was obtained with one of the lentiviral vectors. Work with multigenic vectors carrying alternative promoters (CMV and GRE5) is continuing in the sheep.

The chief investigators acknowledge the contributions of Dr Douglas Parker, Ms Sarah Brice, Ms Lauren Mortimer, Mr Yazad Irani and Ms Alison Clarke to all of this work.

Publications

Williams KA, Brereton HM, Coster DJ. Prospects for genetic modulation of corneal graft survival. *Eye* (Lond) 2009; 23: 1904-9.

Parker DG, Brereton HM, Coster DJ, Williams KA. The potential of viral vector-mediated gene transfer to prolong corneal allograft survival. *Current Gene Ther* 2009; 9: 33-44.

Parker DGA, Brereton HM, Klebe S, Coster DJ, Williams KA. A steroid-inducible promoter for the cornea. *Br J Ophthalmol* 2009; 93:1255-9.

Williams KA, Coster DJ. Gene therapy for diseases of the cornea. *Clin Experiment Ophthalmol* 2010; 38: 93-103.

Parker DG, Coster DJ, Brereton HM, Hart PH, Koldej R, Anson DS, Williams KA. Lentivirusmediated gene transfer of interleukin 10 to the ovine and human cornea. *Clin Experiment Ophthalmol* Feb 2010. [Epub ahead of print].

The effect of hyperglycaemia on experimental glaucoma

A/Prof R Casson and Prof P Blubergs

ORIA Young Investigator/RANZCO Eye Foundation Grant

Aims

We have previously shown that short-term hyperglycaemia can protect against ischaemic retinal injury. From population-based studies we know that there is only a weak association between diabetes and glaucoma. Given that diabetes is a microangiopathy and there is considerable evidence that the pathogenesis of glaucoma involves perfusion problems at the optic nerve head and retina, one could expect a higher association between the two conditions. The aim of the current project was therefore to investigate whether hyperglycaemia is also neuroprotective in a rodent model of experimental glaucoma.

The models

To test the above hypothesis we established a commonly used rat model of ocular hypertension. The model is based on laser application to the trabecular meshwork, which causes scarring and outflow obstruction, resulting in sustained elevation of the intraocular pressure for 2–3 weeks. Over the last two years, we have performed a spatiotemporal characterisation of the pattern of optic nerve injury that occurs following elevation of the intraocular pressure. We used the information gained from these studies to test whether raised glucose is neuroprotective to the optic nerve during experimental glaucoma. In order to produce a sustained increase in glucose availability, one group of rats was made hyperglycaemic by injection of a substance toxic to pancreatic islet cells, which results in an insulin deficit mimicking type-1 diabetes mellitus. Induction of ocular hypertension by laser photocoagulation was then performed. A second group

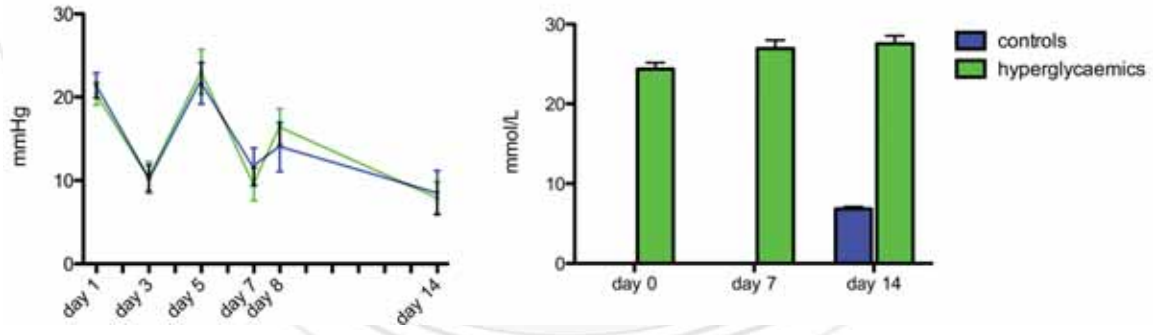


Figure 1: Left: Profile of elevated intraocular pressures in lasered eyes. Data are shown as the mean difference in pressure between the lasered and the non-lasered eyes in each experimental group (n=26 for each group). Right: Mean serum glucose levels for each experimental group.

of rats, which did not have any metabolic disturbances, were also made ocular hypertensive. Elevation of intraocular pressures and serum glucose levels of the two cohorts of rats are shown in figure 1.

Results

1. Injury produced by the glaucoma model

We have shown that the elevated intraocular pressure produces an insult at the optic nerve head region at an early time point. This primary insult is characterised by disruption of axonal transport in axons entering the optic nerve. Almost simultaneously, evidence of axonal cytoskeletal degeneration appear in the corresponding areas. Subsequently, the distal portion of the axons can be seen to degenerate. In addition, proliferation of glial cells and commencement of phagocytosis occurs with ensuing fibrosing of the optic nerve tissue. Over a two-week time course, the model produces a loss of axons of about 20%.

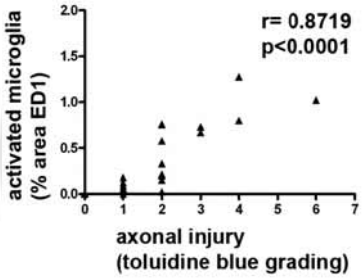
2. Susceptibiliy to injury of different rat strains

Due to external circumstances, we were obliged to use Sprague-Dawley rats from two different sources. We observed marked differences in the susceptibility of the different strains to the laser-induced glaucomatous injury. Attempts to find out more about the determining factors are currently underway.

3. Activation of microglia

We have performed an extensive characterization of the microglial response in our rat model of experimental ocular hypertension. Microglia play a central role in a number of chronic neurodegenerative conditions of the central nervous system, including among others Alzheimer's disease, Parkinson's disease and multiple sclerosis. Their role in these pathologies is not yet clear. One hypothesis proposes that in early stages of disease moderate activation of microglia contributes to enhance survival and regeneration, but in an overactivated, chronically dysregulated state, microglia exacerbate pre-existing damage and contribute to secondary disease progression. The role of microglia may, however, depend on the type and severity of injury. It is therefore very important to study the role of microglia in experimental models of glaucoma (and in post-mortem glaucomatous human tissue).

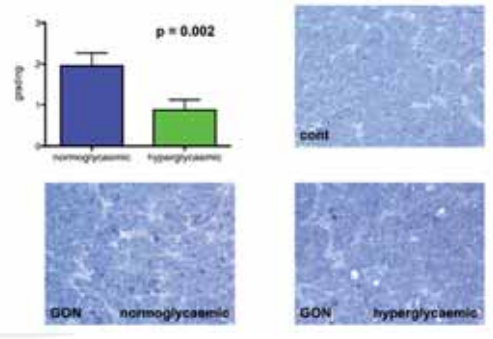
We observed that microglial activation is one of the earliest signs of injury in experimental glaucomatous optic neuropathy. Changes in morphology of microglia can be found as early as 24 hours after the insult. Activation of microglia, which is characterized by morphological changes and an increase in the number of cells, occurs simultaneously along the entire optic pathway to the superior colliculus. Microglia also upregulate multiple immuno-logical cell surface markes.



Furthermore, our studies revealed that certain immunohisto-chemical markers of microglial activity are useful tools to quantify axonal damage. ED1, a lysosomal marker of phagocytic activity, was proven to be of particular interest in the glaucoma model. An excellent correlation was observed between ED1 immunoreactivity and the axonal dmage grade based on assessment of semi-thin optic nerve transverse sections. This is unsurprising, as ED1 is frequently used in models of brain injury as a marker of white matter damage.

4. Protective effect of hyperglycaemia

We found that hyperglycaemia significantly protected optic nerve axons from degeneration in the laser-induced model of experimental ocular hypertension. Axonal loss was reduced by approximately 50% at the two week time point based on semi-quantitative measurement of axonal loss (see figure at right). In agreement with these results, microglial activation (markers used were iba1, a global marker for microglia, and ED1, a marker for phagocytosis) was also shown to be less



marked in the protected group, and expression of stress proteins by optic nerve astrocytes and oligodendrocytes was less pronounced (data not shown).

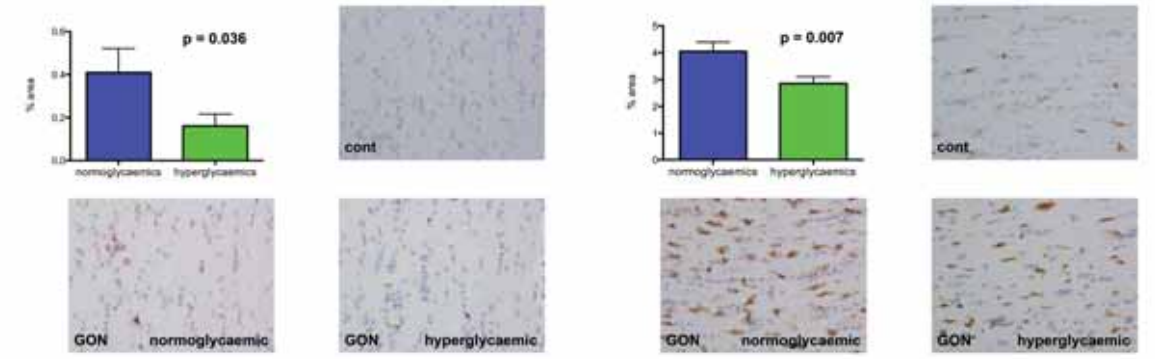


Figure 2: Left: Immunoreactivity for ED1, a marker for activated, phagocytic microglia. Right: Immunoreactivity for iba 1, a marker for microglial proliferation. For each marker, representative images are shown for control nerve (top image), glaucomatous optic nerve from the normogycaemic group (left image), and glaucomatous optic nerve from the hyperglycaemic group (right image).

Future directions

These results have led to the development of a trial of topical glucose as a therapeutic agent in human glaucoma. This proposal was submitted to the ORIA for assessment in 2010.

Opa-1 processing and mitochondrial defects in glaucoma

A/Prof I Trounce and Prof J Crowston

ORIA Grant

Background and aims

Abnormalities in the energy generators of cells (mitochondria) are increasingly thought to play an important role in degenerative diseases of the optic nerve. This project aimed to look for specific abnormalities in the mitochondria of glaucoma patients and age-matched controls. Determining what makes the optic nerve vulnerable in glaucoma will provide a focused approach to developing new treatments.

Autosomal Dominant Optic Atrophy (ADOA) and open angle glaucoma are optic neuropathies that share certain similarities in their clinical phenotype and are both characterized by the specific loss of retinal ganglion cells. Mutations causing haploinsufficiency of Opa-1, a mitochondrial dynamin GTPase, are the most frequent cause of ADOA. Mitochondrial DNA mutations and reduced ATP production have recently also been reported in peripheral blood cells of open angle glaucoma patients.

The specific aims of this study were to:

1. determine whether the Opa-1 isoform profile is altered in peripheral blood lymphocytes of glaucoma patients, either spontaneously or in response to a mild oxidative phosphorylation (OXPHOS) insult
2. determine OXPHOS enzyme-linked ATP production in glaucoma patient mitochondria.

Results

The first achievement of this grant was seeding the establishment of a lymphoblast cell line bank from glaucoma patients. To date we have archived over 30 cell lines, aiming to expand this collection to over 100

patients in the next year. For the present studies up to 15 cell lines were used in different experiments, together with 20 control lymphoblast lines.

Aim 1

Investigation of the Opa-1 protein isoform profile: Figure 1a shows an example of a western blot of lymphoblast cell proteins probed with an antibody to Opa-1, with the 5 known length isoforms clearly resolved in this high resolution gel. Figure 1b shows the quantification of Opa-1 protein levels for all isoforms, where no difference was seen compared to controls. Further analysis of each isoform, and analyses of Opa-1 profiles following growth of cells in galactose (which forces aerobic ATP supply via mitochondrial OXPHOS) also showed no differences between glaucoma patients and controls (not shown). In conclusion, while the experiments were completed at the expected standard, no differences in Opa-1 processing could be identified to distinguish glaucoma patients from controls.

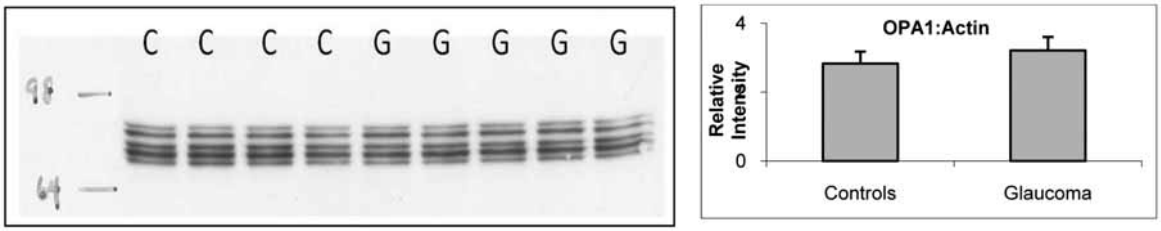


Figure 1a (left) shows the Opa-1 protein clearly resolved into 5 length isoforms. 'C'=control lymphoblast, 'G'=glaucoma patient lymphoblast. Figure 1b (right) shows the quantification of the western blot signals from a total of 9 glaucoma patients and 8 controls. There was no significant difference in total Opa-1 levels or levels of individual length isoforms.

Aim 2

We next investigated OXPHOS protein levels and function in glaucoma and control cells by western blotting. Overall levels of complexes I through V were not significantly different, but when grown in galactose medium there was significant upregulation of all complexes with a trend to higher levels again in the glaucoma patients (Figure 2). This new finding indicates that OXPHOS regulation can respond to changes in fuel availability, but also suggests that the glaucoma patient cells needed to upregulate this pathway more than control cells, suggesting a mild OXHOS impairment.

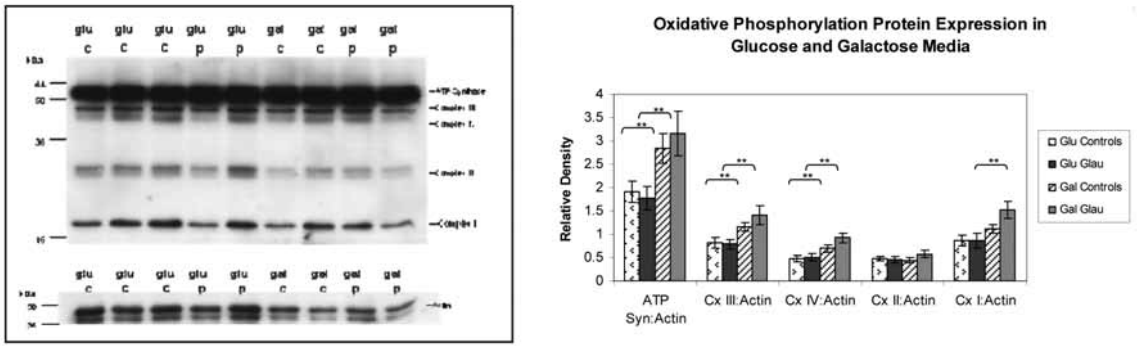


Figure 2. The panel at left shows a western blot example of lymphoblast proteins after growth in either glucose or galactose media. C=controls and P=glaucoma patients. The immunoreactive OXPHOS proteins are indicated. At right is the quantification of the blots from 9 glaucoma patients and 10 controls for each group.

We further investigated OXPHOS function in the cell lines, assaying the maximal rate of OXPHOS-linked ATP production. In Figure 3 the data is shown for substrates that are oxidized via complex I, compared with substrates oxidized via complex II. The results shown are combined from four independent replicates of the glaucoma (n=9) and control (n=10) groups. The complex I-linked ATP production was significantly impaired in the glaucoma group, being 35% lower than controls, while the complex II-linked

ATP production rate trended lower but was not significantly lower. This is an exciting finding. It suggests a mild impairment of complex I, the same enzyme complex which is impaired in the mitochondrial DNA-linked optic neuropathy Leber's Hereditary Optic Neuropathy.

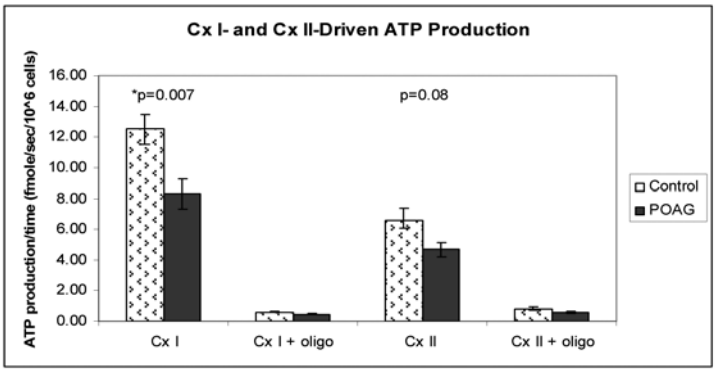


Figure 3. OXPHOS linked maximal ATP production rate in glaucoma (POAG) and control lymphoblasts. The '+oligo' rates indicate the presence of oligomycin, an inhibitor of complex V (the H+ATPase) that demonstrates the specificity of the assay. Significantly lower complex I-linked rates in the glaucoma group suggest a mild impairment of this OXPHOS complex.

In summary, in this ORIA-funded pilot study, we have established strong evidence for a mild but significant defect of complex I-linked ATP production in mitochondria of glaucoma lymphoblast cells. We are currently preparing a manuscript detailing these results. This exciting finding will form the basis of further grant applications, including an NH&MRC application. With further work, we may succeed in defining a sub-group of glaucoma patients that may benefit from new therapies aimed at improving mitochondrial function.

High resolution genomic techniques for novel gene identification in glaucoma

Dr R Jamieson and Dr J Grigg

ORIA/Renensson Bequest Grant

The Eye & Developmental Genetics Research group is investigating the underlying genetic causes in condition that cause blindness in humans. This knowledge provides the foundation for the development of improved treatments and prevention of vision loss. A number of strategies are used to identify disease genes in vision disorders. These include the study of patients and families with ocular disease where there may be chromosomal anomalies, families which are suitable for linkage analysis, and animal models of ocular disease processes.

Glaucoma is the leading cause of irreversible blindness in the world and it affects children and adults. There is a familial predisposition but the underlying genetic causes in glaucoma are mostly unknown. In this project, we are applying the use of recent technological advances in genetic investigation to lead to discovery of novel genes in glaucoma. High resolution comparative genomic hybridisation (CGH) on genomic DNA is used in anterior segment dysgenesis/glaucoma patients to detect regions of microdeletion/microduplication. Fluorescent *in situ* hybridisation (FISH) is used to confirm these regions, and identify chromosomal breakpoints in translocation patients. The CGH microarray and FISH studies lead to accurate localisation of microdeletion/duplication regions, and this allows identification of genes for analysis of expression patterns and predicted functional effects. Genes are selected for characterisation on the basis of their localisation within or immediately flanking deletion, duplication and/or translocation region/s. These genes are analysed using bioinformatic resources to determine their known expression patterns, the encoded proteins' domains and structure, the proteins' functions and cellular localisations. From this project, three strong novel candidates in anterior segment dysgenesis/glaucoma are currently under investigation. These investigations include detailed expression analyses in the anterior segment of the eye, mutation analyses in other human patients with these disorders, *in vitro* assays in cell lines and functional studies in animal models.

From our previous investigation of a chromosomal anomaly patient, we have identified a novel gene, Twist2, which is critical for normal corneal keratocyte proliferation. Twist2 is a member of a family of bHLH transcription factors important in mesenchymal proliferation and differentiation. Our work has shown that Twist2 is expressed first in the periocular mesenchyme and subsequently in the corneal stroma and endothelium of the developing eye. Our examination of a loss-of-function Twist2 mouse model, has shown corneal thinning and a reduced population of stromal keratocytes. We have traced the reduction in the stromal cell population to early stages during which the proliferation of stromal progenitor cells is impaired, and to the reduced number of proliferating cells in the corneal limbus. This is the first time that such a mechanism concerning a mesenchymal transcription factor has been identified and this work is now to be published in Investigative Ophthalmology and Visual Science[1]. This work was also recently presented at the annual meeting of the Australian and New Zealand Society for Cell and Developmental Biology as part of ComBio2009[2]. Support from the ORIA is gratefully acknowledged in publications and presentations of this work.

Publications

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2 Weaving L, Mihelec M, Tam P, Jamieson R. Twist2 is required for normal proliferation of presumptive and limbal corneal keratocytes. ComBio2009, Christchurch, New Zealand, December 2009.

Presentations

- Greenlees R, Storen R, Chen Y, Tam P, Jamieson RV. Wnt signalling and eye development. Westmead Association Research Symposium, August, 2009.
- Ng WY, Wilson M, Grigg J, Jamieson RV. Developmental ocular abnormalities and different etiologies in the one family. Westmead Association Research Symposium, August 2009.
- Jamieson RV. Do genes matter in eye disease? Paediatric Eye Health Summit, Eye Foundation, Royal Australian and New Zealand College of Ophthalmology, Sydney March 2010.
- Jamieson RV. Therapy in Retinal Disease. Retinal Disease Conference, Sydney Eye Hospital, January 2010.
- Jamieson RV. Genetic studies in microphthalmia, anophthalmia and coloboma. Royal Australian and New Zealand College of Ophthalmology Annual Meeting. Brisbane, November 2009.

Development of an artificial silk membrane for retinal pigment epithelial cell growth

A/Prof A Kwan, Prof T Chirila and A/Prof D Harkin

ORIA/Vision Australia Inc Grant

Background

This project addresses the growing interest in techniques for treating retinal disease and especially that related to dysfunction of the retinal pigment epithelium (RPE) in our ageing population. The ultimate goal of the research is to develop improved techniques for growing new RPE cells in the laboratory with the view to subsequent transplantation. One of the key challenges associated with this work is the development of a substrate on which to grow the RPE cells while in the laboratory and which can subsequently be used as a

carrier during transplantation back into the body. In preliminary studies we had demonstrated that a commercially available cell line derived from human RPE cells (ARPE-19) can be grown on transparent membranes produced from fibroin – a fibrous protein isolated from silk which by virtue of being present in silk sutures has had a long history of clinical use and thus is likely to be well tolerated when implanted into the eye. Our initial aims were to further optimise attachment of RPE cells to fibroin membranes and to evaluate the effects of this material on RPE morphology. Funding received from ORIA however enabled us to leverage additional funds from the Prevent Blindness Foundation (Queensland) which in turn enabled us to expand the number of experimental aims to include: modifications to the fibroin membrane structure to improve permeability and hence improved ability to support movement of nutrients and waste products following transplantation, establishment of techniques for growing RPE from donor human tissue, and evaluation of donor RPE cell responses to cultivation on fibroin membrane. We are pleased to report that significant progress has been made in addressing these experimental aims as summarised below.

Progress

1. Optimisation of RPE cultivation techniques

While the ARPE-19 cell line is highly regarded as a suitable model of normal RPE function, care must be taken to grow these cells in the correct way in order that they will develop a morphology resembling that displayed by RPE cells *in vivo*. The principal considerations required to achieve this result are cell density, time in culture and use of low serum concentrations in the culture medium. Over the last 12 months, careful use of these parameters has enabled us to produce cultures of RPE cells from the ARPE-19 cell line which now more closely resemble the normal epithelial structure observed *in vivo* (refer to Fig 3a). Ultimately, our research will need to be repeated using cultures of RPE cells freshly isolated from donor human tissue since this source is likely to best represent the behaviour of RPE cells in patients. It must be said that growing human RPE cells from adult donor tissue is a challenging task owing to a number of logistical and technical issues. Nevertheless, over the last 9 months we have methodically explored a number of published methods and have now achieved a fair degree of success using a technique developed by the Engelmann's group in Dresden (Engelmann and Valtink 2004; Valtink and Engelmann 2009). The key components to success in our hands time that tissue has been stored prior to cell isolation and using a combination of enzymes during tissue digestion as outlined in the group's reports. An example of cultures produced using this approach is shown below in Figure 1.

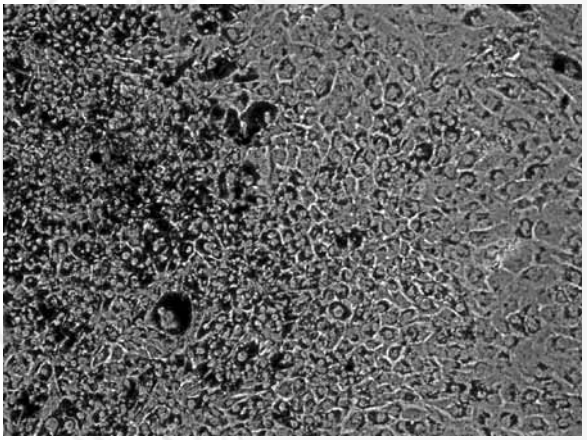

Figure 1: Culture of donor RPE cells derived from cadaveric tissue established using techniques developed by the Engelmann's group. On the left hand side of the image can be seen some smaller cells that still retain a large amount of melanin pigment. Cells towards the right hand side of the picture have begun to lose pigment as has been shown previously to occur during cultivation *in vitro*.

2. Modification of fibroin membranes to facilitate permeability

In preliminary studies, we had used fibroin membranes of between 50 to 100 microns in thickness which while supporting the attachment and growth of RPE cells *in vitro* was considered to be too thick to be used *in vivo*. Moreover, observations made during processing of the fibroin membranes for routine histological studies suggested that the membranes would not be sufficiently permeable to allow efficient movement of nutrients and waste products following transplantation. Hence a formulation of 'porous ultra-thin' fibroin membranes was developed by drying 3–5 micron thick films of fibroin solution (created using a casting

table) which were mixed with a small amount of polyethylene glycol (PEO). During the drying process, the PEG accumulated as localised droplets of approximately 2–3 microns in diameter which could then be subsequently removed by further washing in water resulting in miniature pores across the width of the membrane (Figure 2). Further analysis by scanning electron microscopy combined with routine histological techniques confirms that a significant percentage of the pores traverse the full thickness of the membranes and thus should facilitate free movement of nutrients and waste products. The actual degree of permeability is now being confirmed using a variety of fluorescently labelled molecules of differing sizes that are representative of the molecules that would need to cross the membranes *in vivo*.

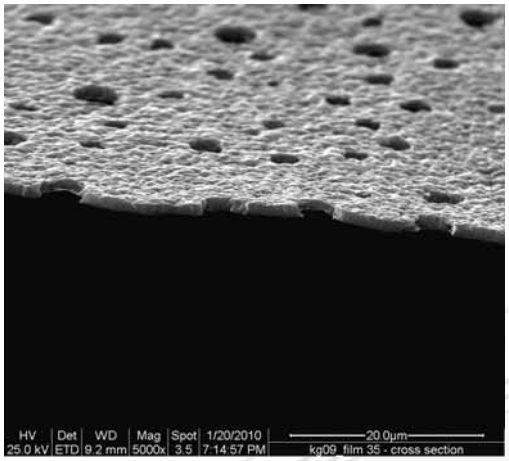

Figure 2: Scanning electron microscopy image of our 'porous ultra-thin' fibroin membrane which we hypothesize will facilitate better function of transplanted RPE cells through enabling more efficient movement of essential nutrients and waste products between the retina and underlying choroidal blood vessels.

3. Assessment of RPE morphology on fibroin membranes

Within the field of RPE research, it is widely accepted that RPE cells grown in the laboratory display a very different morphology to that present in the body unless careful consideration is given to the culture conditions. Moreover, any RPE culture that is designed for transplantation should be carefully evaluated to ensure that it displays what is often referred to as a "polarised" morphology. The conditions optimised in section 1 have therefore been applied to cultures grown on our porous ultra-thin fibroin membrane. Importantly, these cultures have been grown in parallel with those on conventional tissue culture plastic. Thus far, our studies of RPE phenotype using markers for actin filaments and tight junctions indicate a high degree of similarity between the cultures (Figure 3) and further experiments in progress using additional markers (antibodies to keratin 8/18 and RPE-65) and transmission electron microscopy will soon reveal further details on the degree of culture differentiation and polarity that has been achieved. Preliminary results obtained using RPE cultures derived from cadaveric donor tissue confirm that these cells also attach and grown on fibroin membranes and studies of cell phenotype are in progress.

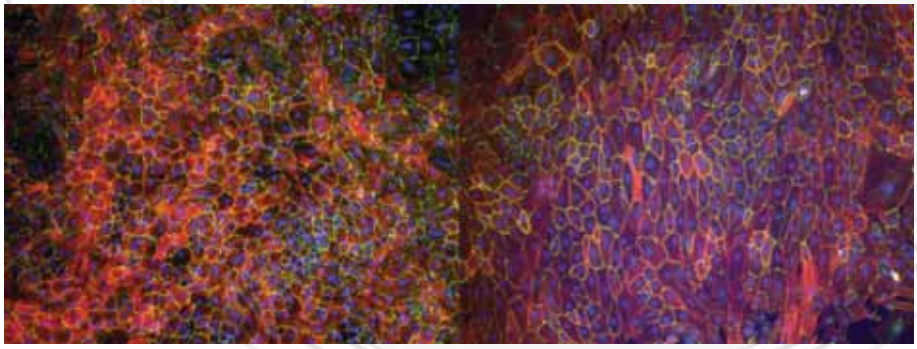

Figure 3: Comparison of long-term cultures of ARPE-19 cells grown on either conventional tissue culture plastic (left) or sheets of porous ultra-thin fibroin membrane. Following nearly two months in culture (necessary to develop epithelial phenotype) the cells were fixed and stained with three dyes to illustrate the presence of nuclei (blue), actin filaments (red) and ZO-1 (green) a protein utilised in the formation of tight junctions between adjacent RPE cells. While some areas in each culture remain poorly organised (absence of green ring around cells) the similarity between cultures is encouraging and indicates that fibroin is worthy of further exploration as a substrate for RPE cell transplantation.

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3. A manuscript detailing the results from studies of ARPE-19 cell growth on fibroin is currently in preparation.

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Glial dysfunction in diabetic retinopathy

Dr W Shen

ORIA Young Investigator grant

In the proposed study, we hypothesized that Muller glial disruption contributes significantly to blood retinal barrier breakdown in retinal vascular diseases including diabetic retinopathy. Experiments have been performed *in vitro* to test the cell specificity of a selective glial toxin, DL-alpha-aminoadipic acid (DL-alpha-AAA), using a number of ocular cells and then we investigated changes in the retinal vasculature after subretinal injection of DL-alpha-AAA in normal and diabetic rats. One conference presentation and one paper have been resulted from this work. In addition, results from this project have led us to extend the study from rodents to non-human primates. This was executed in collaboration with Prof Zhizhong Ma in Peking University Eye Center during 2009–2010. Partial results of this study have been used as preliminary data for an NHMRC project grant application commencing in 2011.

Experiment 1: To test the cell specificity of DL-α-AAA in vitro

First, we investigated the cell specificity of DL-α-AAA on normal human astrocytes (NHAs), rMC-1 Müller cells, bovine retinal vascular endothelial cells (BRVECs), human retinal pericytes and RPE cells *in vitro*. Cell death was observed in NHAs and rMC-1 Müller cells after 16 hr-treatment. With NHAs, 3.5% of cells were killed after treatment with 10mM of DL-α-AAA ($P<0.05$ vs 0mM) but there was no significant increase in the numbers of dead cells after treatment with 0.1, 1 or 5mM of DL-α-AAA. With rMC-1 Müller cells, a significant increase in cell death was observed with a concentration as low as 1mM of DL-α-AAA. Treatment of rMC-1 Müller cells with 1, 5 and 10mM of DL-α-AAA resulted in 7.6%, 95.1% and 94.6% respectively of cells dying. By contrast, there was no evidence of damage to BRVECs, retinal pericytes and RPE cells after 16 hours of continuous exposure to 10mM of DL-α-AAA based on observations of cell morphology and cell viability staining. In addition, changes in cellular metabolic activity were quantitatively measured using the Alamar blue assay. With NHAs, a significant inhibition of cellular metabolic activity was observed with 10mM of DL-α-AAA but not with 0.1, 1 and 5mM concentrations. The cellular metabolic activity of rMC-1 cells showed a concentration-dependent reduction after treatment with 1, 5 or 10mM of DL-α-AAA. There was no reduction in cellular metabolic activities in BRVECs, pericytes and RPE cells

when they were exposed to 10mM of DL-α-AAA for the same period. Results from this experiment indicate that DL-α-AAA is particularly toxic to glial cells including Müller cells and astrocytes among the types of ocular cells tested.

Experiment 2: To study Müller glial changes after subretinal injection of DL-α-AAA in normal rats

To investigate changes in the retinal glia after subretinal injection of DL-α-AAA, double label immunohistochemistry (IHC) was performed using antibodies against glutamine synthetase (GS) and glial fibrillary acidic protein (GFAP). In normal and PBS injected retinas, the GS antibody labelled Müller cells along their entire cellular extent, particularly on the somata within the inner nuclear layer and their endfeet at the inner and outer limiting membranes. GFAP staining was confined to filamentous structures encircling blood vessels in the innermost retina. At four days after injection of DL-α-AAA, there was a decrease in GS immunoreactivity in the somata and outer endfeet of Müller cells, accompanied by increased GFAP immunoreactivity in the retina. At 14 and 60 days after injection of DL-α-AAA, GS immunoreactivity seemed to have recovered to a certain extent or was even upregulated in some Müller cells but strong GFAP immunoreactivity persisted from 4 to 60 days in the injected area. Results from this experiment suggest that subretinal injection of DL-α-AAA induced long-term disruption of Muller cells in the rat retina.

Experiment 3: To study retinal vascular changes after subretinal injection of DL-α-AAA in normal and diabetic rats

To monitor changes in the retinal vasculature after disruption of Müller cells by DL-α-AAA, fundus fluorescein angiography (FFA) was performed periodically after subretinal injection of PBS or DL-α-AAA in rats. Subretinal injection created a bleb in the superior quadrant of the retina that could be easily identified with fundus photography (Fig. 1A). One day after the injection, there was no obvious leak in the area injected with DL-α-AAA except at the site of needle penetration (Fig. 1B, star). Retinal vascular telangiectasis and leak was observed as early as four days post injection and persisted for at least 8 weeks after a single injection (Fig. 1C-E). Subretinal injection of PBS did not induce obvious retinal vascular leak and RPE disturbance at any time (Fig. 1F). Changes in the blood retinal barrier after subretinal injection of DL-α-AAA or PBS were quantitatively studied by measurement of retinal vascular permeability to fluorescence labelled dextran FD-4 (Fig. 1G). The levels of retinal vascular leakage were shown as a ratio to the averaged values of not-treated retinas. Retinal vascular permeability increased slightly in PBS-injected group but there was no statistical difference compared with the not-treated group. Subretinal injection with DL-α-AAA significantly increased the vascular permeability at 4 and 14 days post injection, respectively (Fig. 1G, *$P<0.01$ and †$P<0.05$, $n=5\sim10$ in each group).

To correlation of retinal vascular changes with glial disruption after DL-α-AAA injection, retinal wholemounts were double-stained for isolectin B4 and vimentin to correlate vascular abnormalities with glial disruption after subretinal injection of DL-α-AAA (Fig. 2). In the normal retina, IB4 staining revealed smooth and well-defined retinal vessels, accompanied by even and regular vimentin immunoreactivity (Fig. 2A-D). Subretinal injection of PBS produced slight disturbance of the retinal vasculature, accompanied by increased vimentin immunoreactivity in a limited area around the site of needle penetration (Fig. 2E-H). However, subretinal administration of DL-α-AAA dramatically activated retinal glial cells and disturbed the retinal vasculature, as evidenced by enhanced immunoreactivity of vimentin that was accompanied by prominent tortuosity of retinal capillaries across the injected area, and changes in the retinal vasculature which correlated well with zones of disruption of glial cells in DL-α-AAA injected eyes (Fig. 2I-L).

To test whether Müller glial disruption in diabetic retinas exacerbates blood-retinal barrier breakdown, rats were rendered diabetic by intraperitoneal injection of streptozotocin (STZ, 65mg/kg) or citrate buffer and animals with blood glucose levels>13.8mmol/L were considered as diabetic. Rats received subretinal injection of DL-α-AAA (50μg) or PBS six weeks after diabetes and the BRB was quantitatively measured at two weeks after injection. Our results demonstrated that further disruption of the retinal glia by DL-α-AAA in diabetic rats exacerbated BRB breakdown (Fig. 3, $n=8$ in each group).

In summary, results obtained from this study are consistent with a model in which retinal glial disturbance directly induces abnormalities of the retinal vasculature. In pathological situations like diabetes, further disruption of Müller cells by DL-α-AAA exacerbates breakdown of the BRB. Our findings may be of considerable clinical relevance to understand the molecular and cellular mechanisms of DR in the human retina.

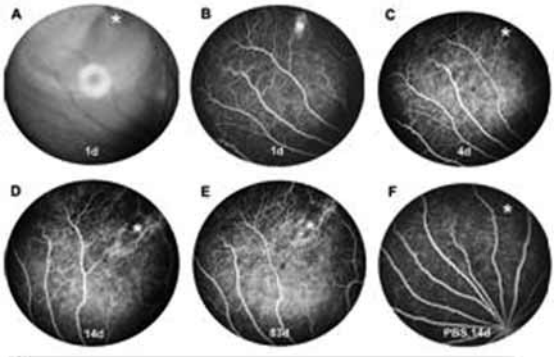

Fig.1

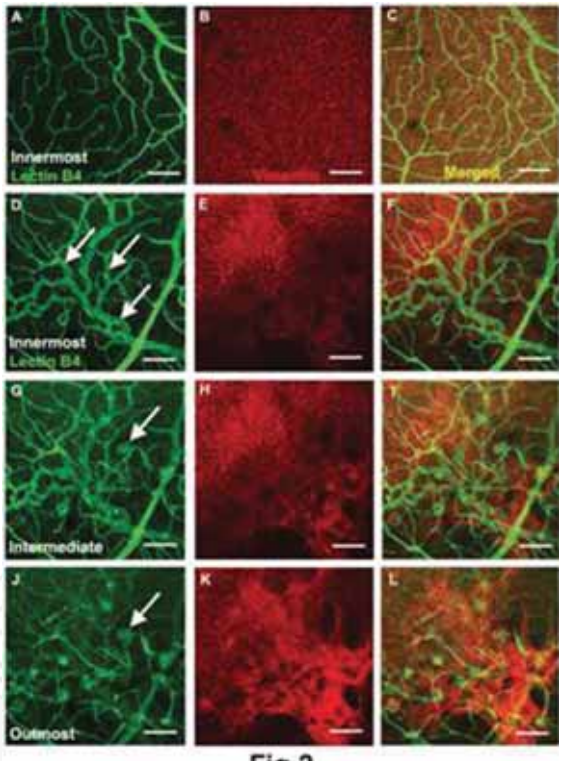

Fig.2

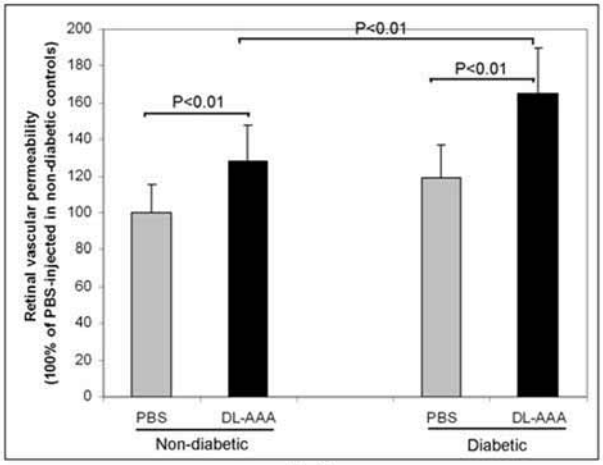

Fig.3

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3. Shen WY, Zhang J, Chung SH, Hu YT, Ma ZZ and Gillies MC. Submacular DL-alpha-aminoadipic acid eradicates primate photoreceptors but does not affect luteal pigment or the retinal vasculature. *Invest Ophthalmol Vis Sci* submitted.

Determining the involvement of non-genetic factors in pseudoexfoliation syndrome

Dr S Sharma, A/Prof JE Craig and A/Prof N Voelcker

ORIA Grant

Pseudoexfoliation (PEX) syndrome is an age-related disorder characterized by accumulation of fibrillar extracellular deposits on the anterior lens capsule. Histologically, pathological extracellular deposits are seen in all tissues surrounding the anterior chamber of the eye. Prevalence of PEX syndrome widely varies in different populations but increases with age. Prevalence of the disease is higher in females than males in some populations. PEX syndrome is the major risk factor for glaucoma, the second leading cause of blindness worldwide. Associated cataract is the most common cause of surgical intervention required by PEX patients. These patients have a higher rate of complications from cataract surgery. Retinal vein occlusion associated with PEX can lead to profound visual loss. Besides ocular pathology PEX syndrome increases the risk of heart disease and stroke. Both genetic and environmental factors are thought to contribute to the disease. Molecular studies to date indicate that oxidative stress and over-production and reduced turnover of extracellular matrix are involved in the disease pathology. However the disease pathogenesis is poorly understood.

Genetic studies have shown that genetic variants of the *LOXL1* (*lysyl oxidase-like 1*) gene are associated with the risk of PEX syndrome in several populations. The product of the *LOXL1* gene is involved in the formation of elastic fibres in tissues that provide resilience to tissues. Thus genetic association of *LOXL1* with PEX syndrome is consistent with the involvement of extracellular matrix proteins in the pathophysiology of the disease. However this genetic association does not explain the variation in disease prevalence in different populations. Therefore additional genetic and/or non-genetic factors are thought to be involved in PEX syndrome.

Epidemiological studies from Australia and India suggest that PEX syndrome is more common in people who experience increased exposure to ultraviolet (UV) radiation from sunlight. UV-A radiation is the major component of sunlight reaching the earth's atmosphere. While the cornea blocks the most of UV-A radiation, some reaches the lens and can induce stress response in the lens epithelial and fibre cells. As the lens epithelium is involved in the production of pathological PEX deposits seen on the anterior lens capsule in PEX syndrome patients, this project aimed to determine, in the lens epithelial cells, the effect of UV-A on expression of those genes implicated in PEX pathophysiology. The study has made considerable progress and the work is ongoing.

Progress to date

For UV-A exposure of cultured lens epithelial cells under aseptic conditions, a UV-A transilluminator was manufactured by the Biomedical Engineering, Flinders Medical Centre. The method for culturing primary lens epithelial cells from lens capsule specimens obtained from patients undergoing cataract surgery was developed. Although growth of lens epithelial cells from the explants in culture was observed from almost every explant (n=12), the cells did not survive sub-culturing, necessary for obtaining sufficient cell numbers for experiments. Hence, primary lens epithelial cultures were found unsuitable for the study. We are instead using an established cell line derived for the human lens epithelium, SRA 01/04, for this study. The SRA 01/04 cells were exposed to various doses of UV-A, and cell viability assayed at multiple time points after treatment. Untreated cells were used as control. A single dose of 6.3 J/cm^2 UV-A resulted in 80% viable cells after 6, 16 and 24 hours post-treatment. Contrarily, repeating treatment with the same dose of UV-A on consecutive days significantly reduced cell viability. Therefore a single treatment with 6.3 J/cm^2 of UV-A is being used as the optimum exposure for SRA 01/04 cells. The SRA 01/04 cells treated with this optimum UV-A exposure and untreated cells have been collected 6 hours after the treatment for RNA extraction. RNA from three replications of treated and untreated SRA 01/04 cells will be used for gene expression analysis. For gene expression analysis, microarray analysis will be performed on the Illumina platform using the Human WG-6 v3.0 Expression BeadChip array at the Australia Genome Research Facility. This expression array includes more than 27,000 reference sequence RNA, more than 7,000 annotated mRNA and more than 12,000 Unigene contigs. Instead of determining the effect of UV-A exposure on expression of only those

genes thus far implicated in PEX, its effect on the entire human transcriptome will be determined to gain a complete understanding of its influence on the genes expressed in the lens epithelial cells. The genes differentially expressed between treated and untreated cells will be validated by quantitative real-time RT-PCR (reverse transcription-polymerase chain reaction). Furthermore, suitable housekeeping genes for use in quantitative real-time RT-PCR in the validation phase of the study have been selected. Six housekeeping genes, namely, *ACTB* (Beta-actin), *GAPDH*, *HPRT1* (hypoxanthine guanine phosphoribosyl-transferase 1), *TBP* (TATA binding protein), *UBC* (ubiquitin C) and *HBMS* (hydroxymethyl-bilane synthase) were amplified from cDNA from SRA 01/04 cells initially by end-point PCR to test the specificity of primer pairs and optimise PCR conditions for amplification. Subsequently, real-time RT-PCR was performed on cDNA from SRA 01/04 cells using the gene-specific primers for each housekeeping gene and SYBR green Mastermix. Specificity of the PCR products was confirmed by melt-curve analysis and agarose gel electrophoresis. Real-time PCR has been optimised for two of the six housekeeping genes, namely, *ACTB* and *GAPDH*. Quantitative real-time PCR will be performed to determine the stability of expression of these housekeepers in UV-A treated and untreated SRA 01/04 cells. These housekeeping genes will be used in the validation phase of the study.

This study is expected to reveal the genes whose expression is influenced by UV-A exposure in lens epithelial cells. It is also expected to reveal the effect of UV-A exposure on expression of those genes already implicated in PEX syndrome.

Refinement of putative loci predisposing to glaucomatous optic neuropathy

Dr A Hewitt

ORIA Young Investigator Grant

Glaucoma is the most common cause of optic neuropathy worldwide and if detected early, blindness is preventable. The aims of this project were to identifying genetic variants which lead to glaucoma blindness. Individual risk profiling could prevent glaucoma blindness by pre-symptomatic screening followed by tailored therapy after disease is detected. Analogous to fire fighting, just as total fire bans and smoke alarms prevent catastrophic damage, knowledge of the genomic profile of at-risk individuals could ensure that patients receive adequate pre-symptomatic clinical screening and early intervention. Additionally, just as all fires (i.e. oil-based versus electrical versus wood) cannot be extinguished with just with any form of hydrant, it is likely that understanding the molecular mechanisms underpinning a particular patient's disease would allow for the use of individualised medicine.

The candidate gene approach has been the basis for hundreds of genetic investigations. A specific-hypothesis driven approach relies on understanding the biology of the disease and precise gene function. For many complex diseases, such as primary open angle glaucoma, the underlying biology is poorly understood and the number of potential candidate genes is legion. We used a genome-wide association design. Genome-wide association holds great promise, particularly in the case of common complex diseases, because no prior information about underlying molecular mechanisms is required. Unfortunately, the high cost of genome-wide arrays has been the major barrier inhibiting the widespread utilisation of such study design. Well-powered GWA investigations generally cost more than AUS$1.5 million. Equimolar pooling of DNA heralds as the most promising approach

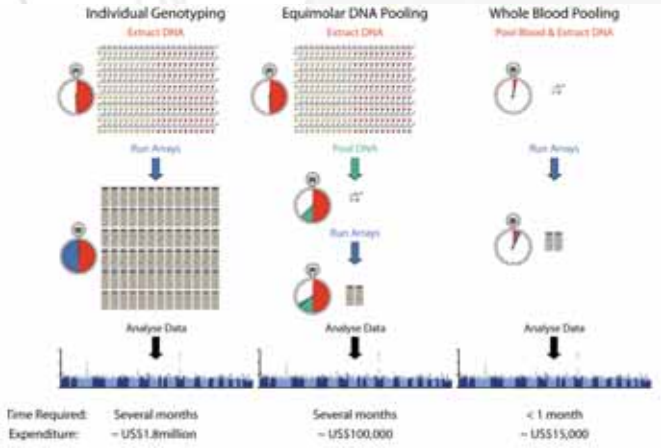

Figure 1.

for cost reduction. In equimolar pooling, rather than individually genotyping each subject in each of the case and controls cohorts, the equimolar DNA pools of all 'cases' and all 'controls' are constructed separately and then genotyped.

ORIA funding allowed us to further explore economical methods for genome-wide association. In particular we showed for the first time that rather than pooling samples at the DNA level, whole blood samples could be pooled allowing for only a small number of DNA extractions and substantial reduction in time required to undertake large-scale gene-discovery investigations. Compared with individual genotyping, pooling can cost 100-fold less.

This novel methodology was utilised to investigate the genomic architecture of OAG and identified a number of putative disease-associated regions. Three case-control cohorts were investigated. Pools were constructed using two independent OAG cohorts – of case subjects with severe glaucoma blindness and age-matched normal controls – from South Australia and Tasmania. The South Australian case cohort represented the first spoke of the Australian and New Zealand Registry of Advanced Glaucoma, whilst the Tasmanian cohort was recruited through the Glaucoma Inheritance Study in Tasmania (GIST). Additional control subjects for the less severe GIST glaucoma cohort were selected through the Blue Mountains Eye Study.

Five SNPs at Xq25 were found to be strongly associated with OAG (χ^2 range: 14.4-22.9). The mean r^2 between the SNPs at this locus was 0.921. The effect at this locus was greater in the subjects who had advanced glaucoma. Following individual genotyping, 18 autosomal SNPs were found to have a significant ($p \leq 0.001$) allelic or genotypic association with OAG. Three of these remained highly significant on joint analysis of the full cohort. Being homozygous for the high risk allele in at least two of these three loci, conferred an OR for developing OAG equal to 2.13 (95%CI: 1.57-2.88; $\chi^2=26.5$, $p=2.64 \times 10^{-7}$), when compared to people who had no high risk allele in the homozygous state. This effect increased to an OR of 3.43 (95%CI: 2.11-5.60; $\chi^2=28.9$, $p=7.62 \times 10^{-8}$) when analysis was refined to solely those cases at the severe end of the OAG disease spectrum.

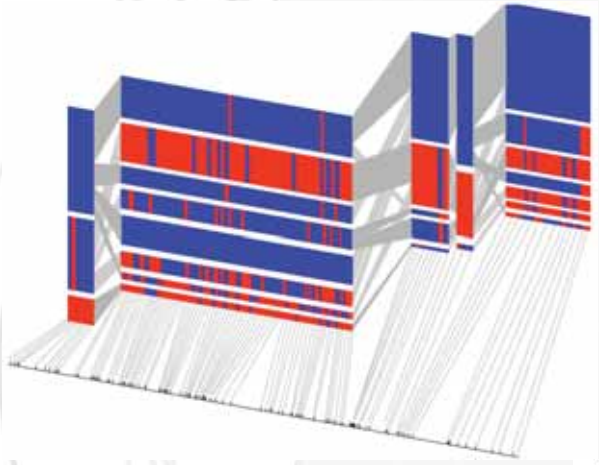

Figure 2. Haplotype Block Structure in the HapMap Centre d'Etude du Polymorphisme Humain population of the novel glaucoma locus on Xp25. Image based on the work of Dr Ben Fry, colours represent allelic variants and the z-offset emphasises the transition between blocks.

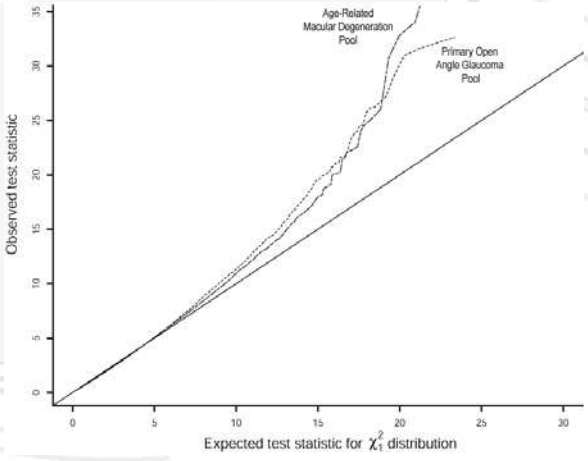

Figure 3. Quantile-quantile plots of test statistics obtained from the genome-wide association of exudative age-related macular degeneration and blind primary open angle case-control DNA pools.

Clearly further work is required before the association of variants at these novel loci with OAG is definitively proven. Replication in additional disease cohorts, in addition to functional molecular studies, will remain essential facets of such work. A caveat on such investigations is that should the true causal variant(s) at any of these loci be identified, then they are likely to have a greater effect size than that observed here.

In summary, ORIA funding allowed the identification of novel putative genetic risk factors for glaucoma through the use of well-characterised ethnically homogeneous cohorts, coupled with the power of high density genetic markers. Our strategy of concentrating analysis on the cases with definitive end-stage glaucomatous visual field loss was chosen to maximize the probability of finding strong novel genetic associations. Additionally, our design of undertaking GWA on a subset of the full cohort is well established. Despite being labour-intensive, there is a very high chance that novel genes accounting for the major common genetic contribution to many inherited human diseases could be uncovered relatively cheaply using equimolar DNA or whole blood pooling.

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The role of tau in experimental glaucoma

Dr J Wood and Dr G Chidlow

ORIA/Glaucoma Australia Inc Grant

Aims

By employing two well-characterised experimental paradigms that relate to glaucoma, we aimed to investigate alterations in tau expression, hyperphosphorylation and aggregation in situations of retinal axonal damage. By doing so we aimed to have a much fuller understanding of the role of tau in such models and be in a better position to relate our findings to glaucoma.

Specifically we aimed to:

1. determine spatio-temporal alterations in tau expression, phosphorylation or aggregation in well-characterised *in vivo* models of experimental retinal ganglion cell death and experimental glaucoma.
2. determine the effects of tau hyperphosphorylation/aggregation on axonal integrity and ganglion cell death using cell culture models.

1. Tau Expression, Phosphorylation and Aggregation in Animal Glaucoma Models

After intraocular injections of N-methyl-D-aspartate, investigations have identified that tau expression levels in the optic nerve remain the same for up to 72 hours, but that this is concurrent with an increased level of (hyper) phosphorylation of this protein at a number of distinct amino acid residues (using a range of relevant phospho-tau antisera). Our analyses have now included immunoprecipitation techniques as an adjunct to the Western blot studies; this improves upon relevant protein yield and enables a cleaner picture to be garnered of the effects of the experimental insults on, specifically, the tau protein species. Exemplified below are the data for the first three days following NMDA injection for the epitope, p^{396}-tau. The marked increase in phosphorylation at this residue is indicative of tau hyperphosphorylation and aggregation events being induced.

Similar results were seen in the laser-induced experimental glaucoma animals, where significant elevations were determined in phospho-tau species known to be associated with neurofibrillary tangle deposits after one to two weeks. Attempts to correlate these findings with immunohistochemistry on retinal/optic nerve sections taken from experimental animals also identified that tau was not only expressed in ganglion cell axons, as was to be expected, but was also clearly present in all oligodendrocyte glia in the optic nerve[1]. We, in fact, identified that tau levels are *increased* in the latter cells at times of more than three days (NMDA animals) and seven days (experimental glaucoma animals) following ganglion cell injury, a finding previously noted only in the brain. We are currently completing our analyses of tau aggregation in the retinas and optic nerves of experimental animals as described in the application which will be concluded soon. Publications are also being prepared.

2. Effects of Tau Alterations on Cultured Ganglion Cells

We have used three models of ganglion cell culture. We have recently characterised that the first, the RGC-5 cell line, does not accurately represent a useful ganglion cell model; in fact this cell line is of an unknown lineage. As an aside, along with the group of Prof Crowston in Melbourne, we have more fully characterised these cells as mouse neuronal precursor cells. Two publications have arisen from these studies[2,3]. We have, moreover, optimised both our mixed (neuron-glial) retinal cell culture system and our purified adult ganglion cell paradigm. In the former system, we have found that tau is labelled in all neurons; in fact tau now represents an extremely useful marker to analyse for pan-neuronal effects within the whole retinal cell population. Tau is also found to be expressed in all cell bodies, dendrites and axons of purified, cultured ganglion cells. In both cases, tau labelling is altered to intense punctate foci within processes, as assessed by immunocytochemistry, when treated with axopathic agents; this occurs prior to axonal degeneration. Studies are currently concentrating on mechanisms and prevention of such events. We have also developed a paradigm of 'chemical anoxia' (i.e. using a known mitochondrial toxin, sodium azide) to induce neuronal distress *in vitro*, due to inconsistencies in our oxygen-glucose-deprivation model. A manuscript has been prepared and submitted for publication, detailing and characterising this new model.

Latter studies have centred on using the PP2A inhibitor, okadaic acid (OA), to *induce* tau hyperphosphorylation in cultured cells. Results using this method have been very encouraging so far and we have been able to show that insults with this compound of only 60 minutes cause a marked increase in cellular labelling for phospho-tau products.

Studies are ongoing and a number of further publications are expected.

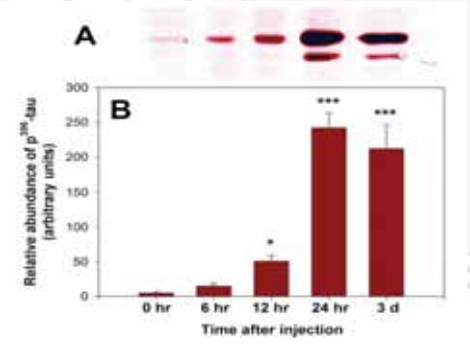

Figure 1: Relative abundance of p^{396}-tau (CST antibody) in rat optic nerves at different times after intraocular injection of NMDA. A, example blots; B, quantification of blot data. Significant elevations of this phosphorylated tau form are present after 12 or more hours. * $P < 0.05$, *** $P < 0.001$; n = 6 samples.

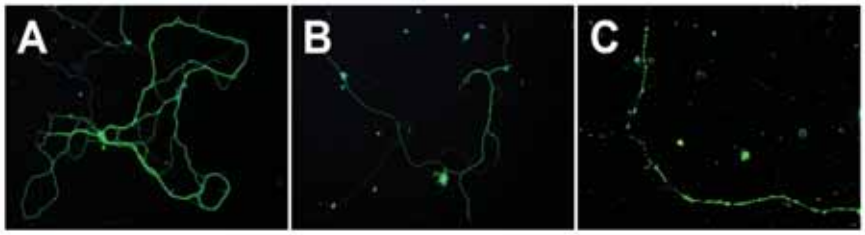

Figure 2: Control labelling of cultured ganglion cells for neurofilament; (B) control cells labelled for tau; (C) cells treated with 10nM OA for one hour and labelled for phospho-tau.

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Complement proteins and photoreceptor death in light-induced retinal degeneration

Dr K Valter-Kocsi, Dr JG Wong, Prof JM Provis and Dr M Madigan

ORIA/Brenda A Mitchell Bequest Grant

Activation and compromised regulation of the complement system are important in the development and progression of age-related macular degeneration (AMD), however it remains unclear as to what initiates these immune processes in the retina. This study aims to investigate if photoreceptor death itself could be the triggering event that activates immune responses, which if not controlled adequately, eventually lead to the development of AMD. We used a light-induced retinal degeneration rat model to assess the relationship between photoreceptor cell death and complement system activation.

To date we have further characterised changes in the rat retina during and following exposure to strong light. The area with the most severe damage ('hot spot') is always found to be at the centre of vision in the rat retina – this may be considered the functional equivalent of the human macula. In this region, the outer barrier structure that protects the retina from the outside environment breaks down following exposure to damaging bright light. Disruption of the barrier allows invasion of immune cells, such as choroidal microglia into the retina. These immune cells are responsible for clean up of damaged cells, but may themselves cause further tissue damage. We observed histological changes in the 'hot spot' following exposure to light that are very similar to those seen in human 'dry' (atrophic) AMD. The results of this study are published and in press[1].

We also investigated how microglia are activated and gather specifically around the 'hot spot' in the retina. Monocyte chemoattractant protein (MCP-1 or Ccl2) was seen in the retina as early as 12 hours after light exposure began which correlated closely with the beginning of large-scale photoreceptor loss. Ccl2 expression continued to increase and by the end of the light exposure period, a significant number of Müller cells, the resident macroglial cells of the retina, showed positive labelling for Ccl2 in the 'hot spot'. Accumulation of microglial cells in the 'hot spot' closely followed the time course of Ccl2 up-regulation. Activated microglial cells were also found to express Ccl2 which can then further attract other immune cells to this location. A manuscript describing the results of this study is in preparation[2].

More recently we found that invading microglia also expressed complement system proteins. The complement system is a key group of proteins that are responsible for the recognition of potentially toxic

agents and the initiation of clean-up of damaged or dead cells within the tissue. Activation of the 'up-stream' members of the complement system can then initiate a cascade of events, which, if not controlled adequately, can lead to further cell loss. We examined the temporal relationship between the activation of genes of the complement system and photoreceptor cell death, and found that complement gene activation closely follows the onset of photoreceptor cell death. When the presence, location and regulation of complement proteins in the retina was studied, we found complement proteins (C1q and C3), in normal healthy retina, located in retinal vessels only. After retinal light damage, expression of complement proteins became more prominent not only in the vessels but also in the retinal tissue itself, mostly in the 'hot spot'.

From these studies, we now have a better understanding of the sequence of events in the development of retinal degeneration, which could be similar to processes occurring in different types of disease, with the common element of the loss of photoreceptors. It seems that exposure to bright light causes photoreceptor damage and loss in the central area of vision, that initiates the up-regulation of a chemo-attractant molecule (Ccl2) in the Müller cells, and signals to microglia cells in the surrounding tissue. This leads to the activation and recruitment of glial cells from the surrounding vessels into the damaged area. Once the barrier protecting the retina is damaged, it allows the large-scale invasion of glial cells, from the choroidal vasculature, into the retina. These cells, apart from cleaning-up the debris in the tissue, also produce complement proteins as well as a chemo-attractant, to recruit even more glial cells. This might be responsible for the close temporal relationship of the up-regulation of complements to photoreceptor cell death.

Recently we found that 670nm near-infrared light is beneficial to the retina. Our data showed that a few short treatments with 670nm light can prevent severe photoreceptor loss and can ameliorate the loss of retinal function in the light-damage rat model. We conducted gene microarray experiments to assess the effects of bright light on the expression of retinal genes and the effects of 670nm light treatment in the healthy control and light-exposed retina. Exposure to bright light induced a 1000-fold increase in Ccl2 gene regulation, and a 2.5-fold increase in C3 gene expression. Treatment with 670nm light, prior to light damage, reduced the expression of both genes by around 50%. The gene microarray project is submitted and under review[3]; the study of the effects of 670nm light treatment on retinal integrity and inflammatory status is being prepared for publication[4].

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Valter, K. Promising approaches to the prevention and treatment of retinal degeneration. Invited seminar at Singapore Eye Research Institute, July 2009.

Valter, K. Light – a double-edged sword: light damage and photobiomodulation. Invited seminar at Center for Complex Systems and Brain Sciences (Prof Janet Blanks, dir), Florida Atlantic University, Boca Raton, Florida, USA, April 2009.

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The association between inflammation and progression of age-related macular degeneration (AMD)

Dr L Robman and Prof R Guymer

ORIA/RANZCO Eye Foundation Grant

A great need for relatively non-invasive diagnostic markers for both AMD progression and measures of the success of intervention has focused AMD research on a search for quantifiable 'biomarker(s)' upon which to measure treatment efficacy.

Genetic and histopathological data on AMD association with inflammation, complement activation and the alternative complement pathway suggested the possibility of using systemic inflammatory biomarkers as possible indicators of the AMD risk by measuring their serum levels.

We have conducted the analysis of the levels of inflammatory biomarkers in serum samples of AMD cases and their controls with no AMD, and also in early AMD cases that progressed in AMD severity. We hypothesized that in subjects with various stages of AMD, one or a combination of inflammatory markers will be significantly elevated in AMD cases compared to controls, and also those who progress in severity of their AMD, when compared to those who do not progress.

1. As a part of the research on the inflammatory markers and AMD, we have tested the influence of C-reactive protein (CRP) levels and of the interaction of CRP levels and complement factor H polymorphism on prevalent AMD and its progression.

   We conducted these tests in a two-arm case-control study: a) on prevalent AMD cases and population-based controls; b) on AMD progression, comparing those in whom AMD progressed to those with no progression.

   In the cross-sectional sample, 312 participants had features of early or late AMD and 232 were controls; in the progression sample, 254 early AMD cases were followed up for seven years.

   The study was conducted in Melbourne. Macular stereo photographs were graded for AMD according to the International Classification and Grading System. High sensitivity CRP was measured in fresh serum, and genotyping was performed through the Australian Genome Research Facility. The association of CRP with outcomes was tested using multivariate logistic regression analysis adjusted for age, smoking, anti-inflammatory medications, and the CC genotype of the CFH gene. Risk factor interaction was explored using an additive model.

Elevated CRP levels were associated with late AMD: odds ratio (OR) 3.12, 95% confidence Interval (CI) 1.38, 7.07. An association of elevated CRP with AMD progression was weaker: OR 1.90, (95%CI 0.88-4.10). A combination of elevated CRP and the CC (*Y402H*) genotype resulted in a super-additivity of the risks, with odds ratios of 19.3 (95% CI 2.8-134) for late AMD, and 6.8 (95% CI 1.2-38.8) for AMD

progression, with the attributable proportion of risk due to CRP-*CFH* interaction calculated at 26%, for prevalent late AMD, and 22% for AMD progression.

Synergistic influence of CRP levels and the at risk genotype of the *CFH* gene resulted in a super-additive risk for prevalent late AMD and AMD progression. Testing for the combination of these two risk factors in order to predict a high risk of AMD and its progression would allow for targeted trials of new intervention strategies.

2. In order to determine the associations of other systemic markers of inflammation with prevalent AMD, we tested serum of 236 patients or visitors of the Royal Victorian Eye & Ear Hospital, Melbourne. Of the total, 42 subjects were controls with no AMD, 46 had early AMD (large drusen), 24 had geographic atrophy (GA) and 124 had choroidal neovascularisation (CNV). Blood draw, clinical examination, and digital fundus photography were conducted and data on demographics and medical history, including the use of anti-inflammatory medication were collected. Blood samples were collected on the day, processed and stored within an hour at -80°C until assayed for a range of systemic markers of inflammation. Investigated inflammatory markers included C-reactive protein, CC chemokine ligand-2/monocyte chemotactic protein-1 (CCL2/MCP-1), soluble intercellular adhesion molecule-1, interleukin-1β, interleukin-2, interleukin-6, tumor necrosis factor-alpha (TNF-α), and transforming growth factor-β1.

   The mean levels of CCL2/MCP-1 were highest in controls (483.3 pg/mL) and lowest in CNV (333.4 pg/mL). The mean levels of IL-6 were lowest for early AMD (1.9 pg/mL) and highest for GA (3.9 pg/mL). The mean level of C3a-des Arg was 10% lower in CNV cases compared to controls (1048.4ng/mL vs 927ng/mL).

   The mean levels of tumor necrosis factor-α (TNF-α) significantly differed between controls and CNV. Lower levels of TNF-α were associated with CNV, with OR = 0.19 (0.06, 0.69). The same tendency was detected for the levels of CCL2/MCP-1. We have not detected association of the other markers with AMD stages in this sample.

These results may indicate that some systemic inflammatory biomarkers are associated with specific subtypes of AMD. We plan to recall those people recruited into the epidemiological study Cardiovascular Health and Age-Related Maculopathy, previously conducted at CERA (2001-02) to determine if any of these biomarkers are associated with progression of AMD in a longitudinal manner. If a serum biomarker can be linked to early disease and the risk of progression of AMD then this would be a significant advance in our ability to manage this disease.

Publication

L Robman, P Baird, P Dimitrov, A Richardson, R Guymer. C-reactive Protein Levels and Complement Factor H Polymorphism Interaction in Age-related Macular Degeneration and its Progression. *Ophthalmology*. Feb 2010.

Presentations

C-reactive Protein Levels and Complement Factor H Polymorphism Interaction in Age-related Macular Degeneration and its Progression. L Robman, P Baird, P Dimitrov, A Richardson, R Guymer. ARVO-2009.

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Variability of Inflammatory Biomarkers in Age-related Macular Degeneration. C Wong, L Robman, J Xie, R Guymer, L Lim. RANZCO – 2009 Congress, Brisbane.

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Central blood pressure and arterial waveform analysis in glaucoma and association with vascular factors

Prof S Graham, Dr M Butlin and Prof A Avolio

ORIA/Esme Anderson Bequest Grant

Overview

The pathogenic mechanisms involved in glaucoma still remain controversial. Elevated intraocular pressure (IOP) is clearly associated but up to a third of glaucoma patients never manifest elevated IOP (normal tension glaucoma (NTG)), and not all patients respond to pressure reduction, with some continuing to progress despite normalised IOP. Vascular mechanisms have been proposed as an additional mechanism to IOP.

While an association between systemic blood pressure (BP) and glaucoma has been established in many incident studies (eg BMES), progressive glaucomatous disease has frequently been linked to systemic hypotension or more precisely to reduced ocular perfusion pressure, supporting the role of ocular blood flow in the pathogenesis[1]. Several recent major long term studies have supported a link between glaucoma and reduced ocular perfusion pressure[2,3]. In studies of systemic hypertension it is now accepted that arterial stiffness, as measured by pulse wave velocity (PWV) and augmentation index (AI), is associated with increased risk of cardiovascular events and mortality. The Rotterdam study (2006) showed that even in apparently healthy people, arterial stiffness remains an independent predictor of heart disease and stroke. The arterial stiffness, or decreased elasticity of vessels, increases the aortic central and pulse pressures, with a greater pressure wave reflection.

The central aortic pressure can be accurately estimated using radial tonometry and applying a transfer function[4]. Central aortic pressure is often lower than brachial BP, which may have implications in disease such as glaucoma where reduced perfusion pressure has been implicated. From the derived aortic waveform an augmentation index (AI) can also be calculated to measure arterial stiffness.

In this study we used the SphygmoCor pulse wave analyser (SphygmoCor, Sydney Aust) to measure brachial BP and radial pulse waveforms, and to then derive a central BP and AI. The waveform can be further studied by determining ratios of systolic to diastolic time and area under the pulsation curve, as indirect markers of perfusion in diastole (eg Form Factor and Subendocardial Viability Ratio – SEVR). We collected individual data on the type of glaucoma (POAG vs NTG), history or presence of disc haemorrhages, peripapillary atrophy, presence of spontaneous retinal venous pulsations (SVP), migraine, smoking history, hyperlipidemia, medications (including statins) and whether the glaucoma has been stable or progressing.

Progress

We examined 126 glaucoma patients (90 POAG and 36 NTG) who were on treatment and who had been followed for at least three years and had several reliable visual fields such that glaucoma progression could be determined on the basis of the Humphrey GPA program. We also tested 50 age and sex matched controls. Data was analysed using multiple linear stepwise regression using blood pressure variables with and without ocular variables in the model.

Overall we found no significant difference between systemic or derived central aortic BP parameters between glaucomas and normals. However there were several findings that emerged from the regression analysis. Brachial pulse pressure was lower in the combined glaucoma group compared to controls ($p<0.003$), and also in the POAG group when analysed separately, but not the NTG group.

In the arterial waveform analysis POAG was associated with a higher SEVR ($p<0.01$) and a lower brachial Form Factor than NTG ($p<0.02$). Disease progression was associated with disc haemorrhages as expected ($p<0.02$) but also negatively associated with spontaneous venous pulsations ($p<0.002$). The latter is in keeping with reports by Morgan et al that SVPs are reduced in glaucoma.

When the ocular parameters (haems, SVP, peripapillary atrophy) were not included in the regression

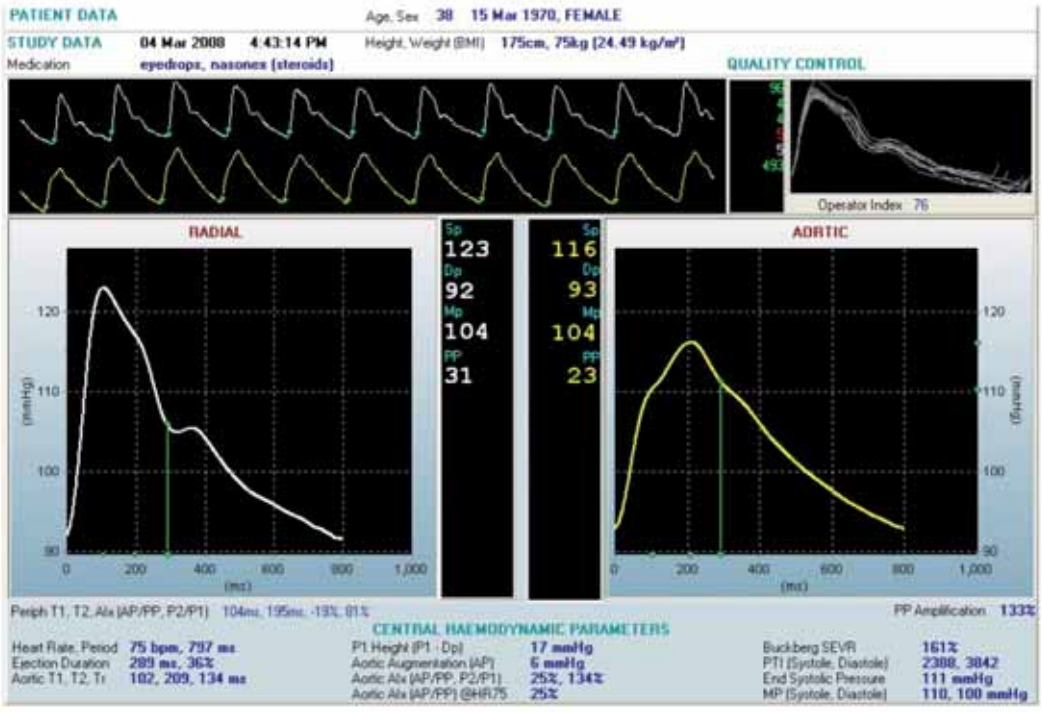

Fig 1. Example of glaucoma patient with reduced pulse pressure and high diastolic BP.

model, the main predictor of disease progression was a decreased SEVR ($p<0.02$). Smoking history also showed a weaker association with glaucoma ($p<0.03$) but not progression.

Since glaucoma pathogenesis is still poorly understood, and there is increasing evidence that vascular factors somehow play a role, this study sought to further examine aspects of blood pressure and pulsatility that might lead to an understanding of mechanism. There does seem to be an association with altered vascular waveforms and reduced pulse pressure, but the study did not show as strong a link with arterial stiffness or central blood pressure parameters as we had hoped. The findings however did support the notion that patients with glaucoma should seek to modify and control factors that can impact on vessel walls such as smoking, hyperlipidaemia and systemic hypertension, as these changes may have a negative effect. The finding that loss of SVPs is a risk factor for progression is novel. The results have recently been prepared for publication.

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The Effects of a Transient Elevation of IOP upon Retinal Function and Structure

Professor G Gole and Dr N Barnett

ORIA Grant

Aims

The mechanisms of glaucomatous damage to retinal ganglion cells are still poorly understood. This project is capitalizing upon three major advances:

1. A clinically relevant, experimental mouse model of glaucoma developed and characterized in our laboratory (Holcombe et al. 2008).
2. The availability of a transgenic mouse that allows longitudinal assessment of ganglion cell survival *in vivo*.
3. An electroretinographic technique for the isolation and analysis of mouse ganglion cell function (scotopic threshold response – STR).

Hypothesis: Some of the intraocular pressure-mediated ganglion cell dysfunction, which precedes ganglion cell death, is reversible.

We are conducting a detailed functional and histological study upon these eyes that have been subjected to a sustained, but short-term, elevation of IOP, followed by a return to sub-threshold IOP (< 22 mmHg).

Methods

Chronic, unilateral, ocular hypertension is induced in our mouse mode (Holcombe *et al*., 2008). A bolus injection of indocyanine green, to maximize episcleral vessel laser absorption, is administered into a tail vein. The episcleral veins adjacent to the limbus are photocoagulated with an 810 nm diode laser. Eighty to 100 spots, size 200 μm, power 100–130 mW and duration 200 ms are applied to the visible vasculature within 0.5–1.0 mm of the limbus around 300 degrees of the eye.

We have now obtained access to a transgenic mouse line incorporating cyan fluorescent protein (CFP) under the control of a promoter for the ganglion cell marker, Thy-1. The retina is imaged *in vivo* using a modified laboratory microscope equipped with epifluorescence. Retinal imaging and quantification of ganglion cells is undertaken longitudinally throughout the transient period of elevated IOP and seven days after the return of IOP to baseline levels (below 22 mmHg), with the contralateral untreated eye as the control. At various time intervals, retinal wholemounts are also prepared for ganglion cell quantification.

ERG Protocol

The scotopic threshold response (STR), reflects retinal activity proximal to the bipolar cells in mice, rats and humans. Animals are dark adapted for 24 hours. Full-field (ganzfeld) ERGs are recorded simultaneously from both eyes. STRs are elicited using a -4.5 log cd.s.m^{-2} stimulus from a Luxeon 1 Watt white LED (5500°K). Thirty flashes with an interstimulus interval of two seconds are averaged. Responses are amplified and recorded with a bioamplifier/analogue to digital converter, band-pass filtered between 0.3 and 1000 Hz, and digitized at 4kHz. Response amplitudes of the STR are measured at 140 and 220 ms after the stimulus flash. These criterion times correspond with the respective STR peaks of averaged control responses obtained previously. ERGs are recorded throughout the transient period of elevated IOP and every other day for seven days after the return of IOP to baseline levels (below 22 mmHg).

Progress to date

The project start was delayed to Feb 2010 due to unforeseen problems with the University of Queensland animal house infrastructure (which necessitated several months closure for urgent repairs and renovation) that prevented breeding of Thy1-CFP mice in Brisbane. All of our newer research techniques are now established in our laboratory. We have employed a research assistant for this project who is now fully trained and competent with all methodologies. We are now on track to achieve all outcomes as proposed, albeit delayed by one year.

Results

A. Successful imaging and quantification of Thy1-CFP-positive Retinal Ganglion Cells post-mortem.

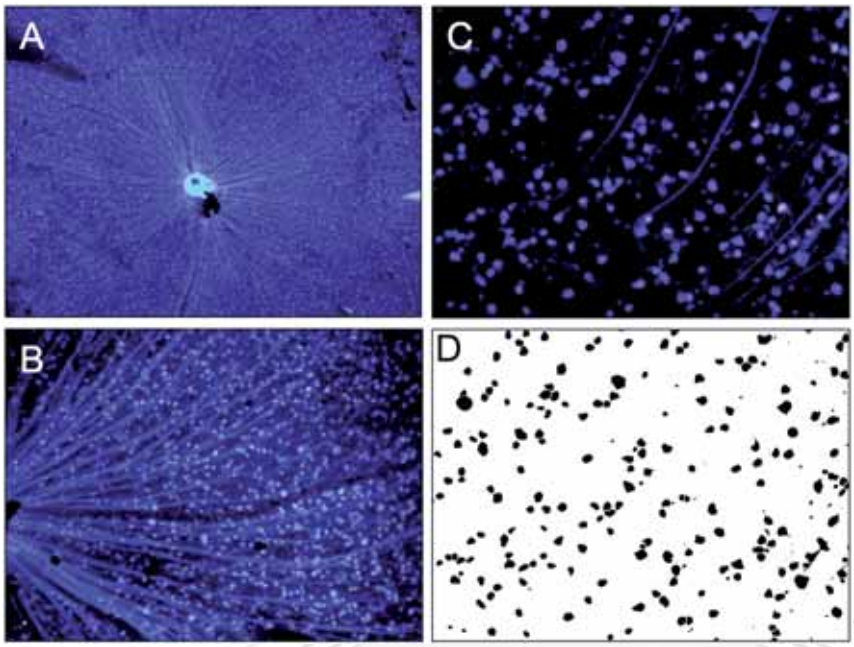

Figure 1. Mouse retina1 wholemounts (A, B, C) obtained from a normotensive control eye showing ganglion cells expressing cyan fluorescent protein. Panel D is a binary image of panel C, allowing quantification of ganglion cells with ImageJ software.

B. Successful imaging of Thy1-CFP-positive RGCs *in vivo*.

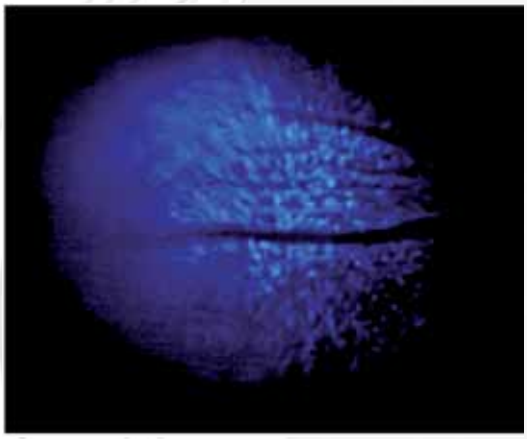

Figure 2. An *in vivo* fundus image of mouse retina showing fluorescent ganglion cells expressing CFP. Refinement of this technique allows us to assess the progression of ganglion cell loss over time following the elevation of IOP, in each individual mouse.

IOP Profile after laser treatment

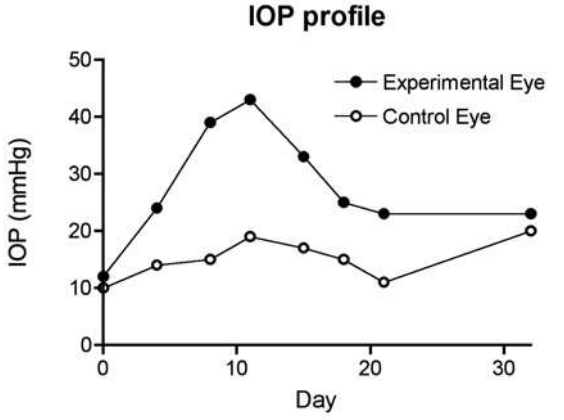

Figure 3. An example of an IOP pressure profile after laser treatment showing a transient elevation of IOP. Transient, rather than sustained, elevation of IOP occurs in approximately 30% of our treated mice.

C. We are yet to determine whether there is a recovery of the positive scotopic threshold response (pSTR) following the transient elevation of IOP. The ERG pSTR is suppressed during the period of elevated IOP. The STR reflects ganglion cell function.

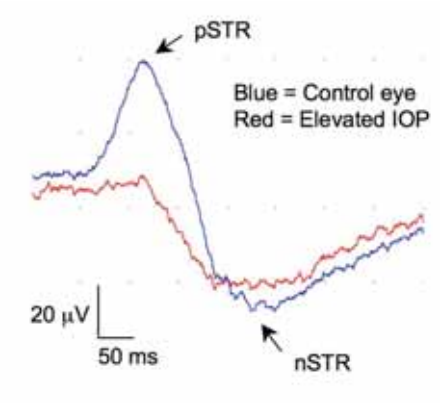

Figure 4. Elevated IOP suppresses the positive scotopic threshold response (pSTR) of the ERG. This example was recorded from a mouse with IOPs of 16 mmHg (control eye, blue trace) and 40 mmHg (lasered eye, red trace). nSTR = negative scotopic threshold response.

D. The histological effects of a transient increase in IOP are specific to the ganglion cells e.g. there is no loss of PKCα-immunoreactive bipolar cells.

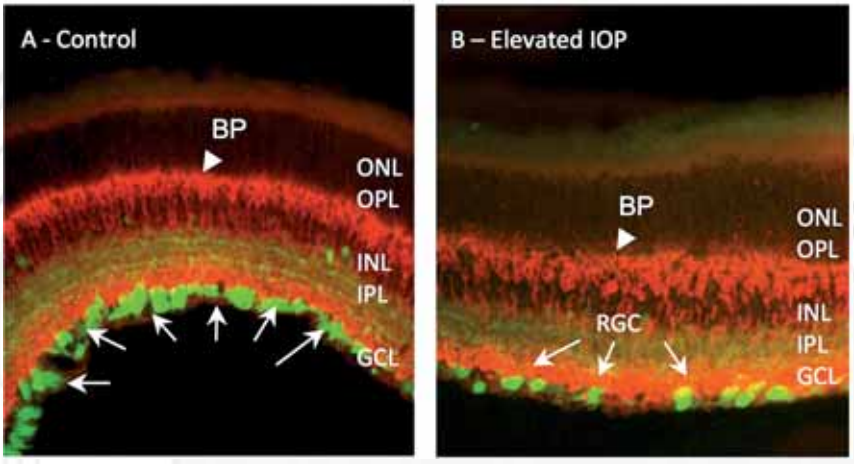

Figure 5. Transverse sections of A) control, and B) experimental mouse retina. A transient elevation of intraocular pressure induces a specific reduction of CFP-expressing retinal ganglion cells (RGC, green). The bipolar cells labelled with PKC (BP, red) are unaffected. Ganglion cell layer (GCL), inner nuclear layer (INL), inner plexiform layer (IPL), outer nuclear layer (ONL), outer plexiform layer (OPL).

Directors' Report

For the year ended 30 June 2010

In accordance with a resolution of the directors, the directors submit herewith the financial statements of The Ophthalmic Research Institute of Australia for the year ended on that date and report as follows:

1. Directors

The names of the Directors of the company in office at the date of this report are:

A/Prof Mark D Daniell, Melbourne (Chairman)
Professor Mark Gillies, Sydney (Vice Chairman)
Professor Stuart L Graham, Sydney (Honorary Secretary)
A/Prof Robert Casson, Adelaide (Honorary Treasurer)
Dr R Max Conway, Sydney
Professor J Crowston, Melbourne
Dr Wilson Heriot, Melbourne
Dr Anthony Kwan, Brisbane
Professor David Mackey, Perth
Professor Peter J McCluskey, Sydney
Dr John Males, Sydney
Dr Richard Mills, Adelaide
Dr Richard J Stawell, Melbourne
Dr Andrea Vincent, New Zealand
Dr Stephanie Watson, Sydney
Professor Tien Wong, Melbourne

2. Information on Directors

The names, qualifications and period membership commenced and position held are as follows:

A/Prof Robert Casson MB BS (Hons), PhD, FRANZCO	Honorary Treasurer	2005
Dr R Max Conway PhD, MD, FRANZCO		2007
Professor J Crowston BSc, MBBS, FRCOphth, FRANZCO, PhD		2008
A/Prof Mark Daniell MB BS, MS, FRACS, FRANZCO	Chair	2001
Professor Mark Gillies MB BS, PhD, FRANZCO	Vice Chairman	2004
Professor Stuart Graham MB BS, MS, PhD, FRANZCO, FRACS	Honorary Secretary	2001
Dr Wilson Heriot MB BS, FRANZCO, FRACS		2009
Dr Anthony Kwan MBChB (UK), MD (London), FRCOphth (UK), FRANZCO		2007
Professor David Mackey MB BS, MD, FRANZCO, FRACS		2005
Professor Peter J McCluskey MB BS FRANZCO, FRACS		1984
Dr John Males MB BS, MMed, FRANZCO		2009
Dr Richard Mills MB BS, FRCS, FRACS, FRANZCO, PhD		2003
Dr Salmaan Qureshi MB BS, FRANZCO		2006
Dr Richard J Stawell MB BS, FRACS, FRANZCO		1984
Dr Andrea Vincent MBChB, FRANZCO		2008
Dr Stephanie Watson BSc, MBBS, FRANZCO, PhD		2006
Professor Tien Wong MB BS, MPH, PhD, FRANZCO		2008
Dr Ehud Zamir MD, FRANZCO (resigned Sept 09)		2007

No Shares are held by Directors.

3. Meetings of Directors

During the financial year three meetings of directors were held. Attendances were:

	Number eligible to attend	Number attended
A/Prof Robert Casson, Adelaide	3	3
Dr R Max Conway, Sydney	3	2
Prof J Crowston, Melbourne	3	3
A/Prof Mark Daniell, Melbourne	3	2
Prof Mark Gillies, Sydney	3	2
Prof Stuart Graham, Sydney	3	2
Dr Wilson Heriot	1	0
Dr Anthony Kwan, Brisbane	3	3
Prof David Mackey, Perth	3	3
Prof Peter J McCluskey, Sydney	3	1
Dr John Males, Sydney	1	1
Dr Richard Mills, Adelaide	3	3
Dr Salmaan Qureshi, Melbourne	1	0
Dr Richard J Stawell, Melbourne	3	3
Dr Andrea Vincent, New Zealand	3	3
Prof Tien Wong, Melbourne	3	3
Dr Stephanie Watson, Sydney	3	3
Dr Ehud Zamir, Melbourne	1	0

4. Indemnifying Officer or Auditor

The company has not during or since the financial year in respect of any person who is or has been an officer or auditor of the company or a related body corporate indemnified or made any relevant agreement for indemnifying against a liability incurred as an officer including costs and expenses in successfully defending legal proceedings or paid or agreed to pay a premium in respect of a contract of insurance against a liability incurred as an officer for the costs or expenses to defend legal proceedings.

5. Principal Activities

The principal activity of the company in the course of the financial period was to provide funds for ophthalmic research. There has been no significant change in the nature of this activity during that period.

6. Operating Results

(1) Operating Revenue

Revenue is mainly derived from investing in shares and interest bearing securities.

	2010	2009	Decrease	%
Net dividend interest and trust distribution income	$500,646	$562,420	$(61,774)	(10.98)
Less Expenses	35,853	33,564		
	$464,793	$528,856		

(2) Operating Surplus

The net surplus of the company before payment of grants for the year ended 30 June 2010 was $686,428 (2009 – $248,213). This amount is comprised of the following:

	2010	2009
Trust Fund	$693,495	$261,661
Administration	(7,067)	(13,448)
	$686,428	$248,213

The net surplus included the profit on re-arrangement of investments amounting to $210,367 (2009 – $373,346 loss), dividend imputation credits amounting to $98,590 (2009 – $123,741) and legacy distributions of $17,345 (2009 – $145,561).

7. Review of Operations

The surplus for the year was $686,428 compared to $248,213 in 2009. The income of the trust fund increased by $438,215 mainly due to a significant gain on the re-arrangement of investments of $210,367 compared to $373,346 loss in 2009. There was a decrease in investment income to $500,646 from $562,420 in 2009 and distributions from legacies and donations decreased to $17,345 from $145,561 in 2009. The administrative operations of the institute for the year resulted in a deficit of $7,067 compared with a deficit of $13,448 in 2009.

8. Dividends

The company's Articles of Association preclude the payment of dividends to any of its members.

9. State of Affairs

There has been no significant change in the state of affairs of the company occurring during the year.

10. Likely Developments

At the date of this report, there are no known unusual developments that will affect the results of the company's operations in subsequent financial years.

11. Share Options

No share options were issued during the year.

12. Directors' Benefits

With the exception of the grants made or allocated to Professor Mark Gillies, Dr Richard Mills, Dr Stephanie Watson, Professor David Mackey, Dr RM Conway and Professor Peter McCluskey, no director of the company has since the end of the previous financial year, received or become entitled to receive a benefit not disclosed in the accounts as directors' emoluments by reason of a contract made by the company or a related corporation with the directors, or with a firm in which he or she has a substantial financial interest.

13. Auditor's Independence Declaration

A copy of the auditor's independence declaration as required under Section 307 C of the Corporations Act 2001 is set out at page 55.

For and on behalf of the Board.

A/Prof M Daniell
Director

A/Prof R Casson
Director

Sydney

Signed in accordance with a resolution of directors,
this 11th day of September 2010

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Balance Sheet

As as 30 June 2010

	Note	2010 $	2009 $
Current Assets			
Cash and Cash Equivalents	3	1,379,928	757,034
Receivables	4	9,333	237,225
Investments	5	7,462,732	7,575,046
		8,939,993	8,569,305
Non-Current Assets			
Plant & Equipment	6	240	384
Total Assets		8,940,233	8,569,689
Current Liabilities			
Payables	7	610,518	543,861
Provision	8	13,307	14,147
		623,825	558,008
Net Assets		8,316,408	8,011,681
Equity			
General Fund	13 (a)	–	–
Capital Funds			
Research Fund	9	917,800	1,053,756
Settled Funds	10	472,556	472,556
Financial Assets Reserve	11	323,934	150,684
Capitalised Profit on Re-arrangement of Investments and Capital Distributions	12	6,283,678	6,073,312
		7,997,968	7,750,308
Retained Income – available for grants	13 (b)	318,440	261,373
Total Equity		8,316,408	8,011,681

The accompanying Notes form part of these financial statements.

Trust Fund Statement of Comprehensive Income

For the year ended 30 June 2010

	Note	2010 $	2009 $
INCOME			
Dividends received from:			
Other corporations		363,718	422,031
Less: Special Dividends and Imputation credits		–	(84,042)
Total Dividends		363,718	337,989
Interest received from:			
Other entities		104,352	165,337
Trust distributions received from:			
Other entities		32,576	59,094
		500,646	562,420
Legacies – Anselmi Estate		12,914	138,954
– Ivy May Stephenson		2,931	6,457
Other donations and legacies received		1,500	150
Sundry Income		990	590
		518,981	708,571
Profit/(Loss) on re-arrangement of Investments		(373,346)	735,855
Total Income from Ordinary Activities		335,225	1,422,001
EXPENSES			
AOVS meeting contribution		–	10,000
Donation – Macquarie University, Chair of Ophthalmology		–	30,000
Commission paid		35,853	33,564
		35,853	73,564
SURPLUS FOR THE YEAR		483,128	635,007
Other Comprehensive Income			
Valuation Gains/(Losses) on available-for-sale financial assets		173,250	(457,104)
Profit/(Loss) on re-arrangement of investments		210,367	(373,346)
Total other comprehensive income		383,617	(830,450)
Surpus for the year before allocation		866,745	(195,443)
Grants allocated/made during the year	14	410,950	376,211
Allocation to Director of Research – Victoria	15	144,000	164,000
		554,950	540,211
TOTAL COMPREHENSIVE INCOME		311,795	735,654
Profit attributable to Members of the Entity		(71,822)	94,796
Total other comprehensive income attributable to Members of the Entity		383,617	(830,450)

The accompanying Notes form part of these financial statements.

Administration Statement of Comprehensive Income

for the year ended 30 June 2010

	Note	2010 $	2009 $
INCOME			
Membership Fees – RANZCO		109,000	98,900
Total income		109,000	98,900
EXPENSES			
Accountancy Fees		19,950	19,000
Auditors' Remuneration	16	4,950	4,950
Bank Charges		138	217
Depreciation		144	230
General Expenses		3,698	1,773
IT and Webpage Expenses		847	715
Insurance		4,122	4,027
Printing and Stationery		9,052	8,366
Staff Salaries		51,182	48,863
Superannuation Contribution		5,326	4,573
Salary Sacrificed Benefits		1,200	1,950
Provision Employee Benefits		(840)	2,020
Meeting and Travelling Expenses		16,298	15,664
Total Expenses		116,067	112,348
DEFICIT FOR THE YEAR	13 (a)	(7,067)	(13,448)
Other Comprehensive Income		–	–
Total Comprehensive Income		(7,067)	(13,448)

The accompanying Notes form part of these financial statements.

Statement of Changes in Equity

for the year ended 30 June 2010

	GENERAL FUND			CAPITAL FUNDS			TOTAL
	Accumulated Surplus/(Deficits)	Research Fund	Settled Funds	Realised Profits on Re-arrangement of Investments and Capital Distributions	Financial Assets Reserve	Retained Income	
	$	$	$	$	$	$	$
Balance at 1 July 2008	–	900,902	472,556	6,446,658	607,788	332,879	8,760,783
Profit for Year	(13,448)	–	–	–	–	94,796	81,348
Total Other Comprehensive Income	–	–	–	–	–	(373,346)	(373,346)
Transfers to/(from) Reserves	13,448	152,854	–	(373,346)	–	207,044	–
Available-for-sale Financial Assets:							
Valuation Gains/(Losses)	–	–	–	–	(457,104)	–	(457,104)
Transferred to Profit for the Period	–	–	–	–	–	–	–
Balance at 30 June 2009	–	1,053,756	472,556	6,073,312	150,684	261,373	8,011,681
Balance at 1 July 2009	–	1,053,756	472,556	6,073,312	150,684	261,373	8,011,681
Profit for Year	(7,067)	–	–	–	–	(71,822)	(78,889)
Total Other Comprehensive Income	–	–	–	210,367	173,250	–	383,617
Transfers to/(from) Reserves	7,067	(135,956)	–	–	–	128,889	–
Transferred to Profit for the Period	–	–	–	–	–	–	–
Balance at 30 June 2010	–	917,800	472,556	6,283,678	323,934	318,440	8,316,408

The accompanying Notes form part of these financial statements.

Cash Flow Statement

for the year ended 30 June 2010

	Note	2010 $	2009 $
Cash Flows from Operating Activities			
Receipts			
Dividends Received		391,710	315,942
Interest Received		104,352	165,337
Trust Distributions		32,576	59,094
Legacies		15,845	33,511
Other Revenue		9,259	740
Membership Fees – RANZCO		109,000	98,900
Contribution from RANZCO		40,000	80,000
Contribution from RANZCO Eye Foundation		75,000	75,000
Contribution from Renensson Bequest		25,000	25,000
Contribution from Glaucoma Foundation		45,791	50,000
Contribution from Vision Australia		–	25,000
Anselmi and Ivy May Stephenson Legacies transferred from capital		111,900	–
Payments			
Commissions		(35,853)	(33,564)
Research Grants Paid		(509,859)	(662,646)
Payments to Director of Research – Victoria		(164,000)	(193,000)
Other Grants and Contributions		–	(40,000)
Other		(123,757)	(108,954)
Net Cash (Used in)/Provided by Operating Activities	17	(126,964)	(109,640)
Cash Flows from Investing Activities			
Proceeds from Re-arrangement of Investments		19,339,296	29,547,289
Payments for Investments		(18,843,366)	(29,930,630)
Net Cash Used in Investing Activities		495,530	(383,341)
Net Increase/(Decrease) in Cash and Cash Equivalents		622,894	(492,981)
Cash and Cash Equivalents at 1 July 2009		757,034	1,250,015
Cash and Cash Equivalents at 30 June 2010	3	1,379,928	757,034

The accompanying Notes form part of these financial statements.

Notes to and forming part of the Financial Statements

for the year ended 30 June 2010

1 Statement of Accounting Policies

The financial statements are for the Ophthalmic Research Institute of Australia, incorporated and domiciled in Australia. The Ophthalmic Research Institute of Australia is a company limited by guarantee.

(a) Basis of preparation

The financial statements are general purpose financial statements that have been prepared in accordance with Australian Accounting Standards (including Australian Accounting Interpretations) and the Corporations Act 2001.

The accounting policies set out below have been consistently applied to all years presented, unless otherwise stated. The financial report has been prepared on an accruals basis and is based on historical costs and does not take into account changing money values or, except where stated, current valuations of non current assets. Cost is based on the fair values of the consideration given in exchange for assets.

The following is a summary of the significant accounting policies adopted by the company in the preparation of the financial report.

(b) Income tax

The company is an approved research institute and is exempt from income tax.

(c) Transfers to Capital Funds

(i) **Capital profits and losses on disposal of investments and capital distributions**

Realised capital profits and losses on disposal of investments are brought to account in the trust fund as profit/(loss) on rearrangement of investments, however, these amounts are transferred to capital funds and do not form part of accumulated income.

Capital distributions and special dividends together with associated imputation credits recognised in the statement of comprehensive income are also transferred to capital fund and do not form part of accumulated income.

(ii) **General Research Capital Fund**

Five percent of the net surplus of the General Fund including imputation credits are transferred to the General Research Capital Fund this financial year.

(iii) **Allocation of income to each fund**

During the year ended 30 June 1993, the investments of the Institute were separated into the D.W. Research Fund and the General Fund in the ratio of 72% and 28% respectively. As the flow of investment and donation income to and from the two funds does not occur in the same proportion, the ratio of the D.W. Research Fund and the General Fund is no longer at 72% and 28%.

Income from the General Fund which comprises all funds except the D.W. Research Fund, is allocated as follows:

Research Fund	10.0%
Esme Anderson	51.4%
G.J. Williams	8.9%
B. Mitchell	8.9%
Dame Ida Mann	12.5%
R. & L. Lowe Research	8.3%

If and when further donations are received by specific fund(s) the allocation of future income will be distributed to each fund in accordance with its revised proportion to the General Fund.

Fifty per cent of the income derived from the D.W. Research Fund and its investments is allocated to the Director of Research Victoria.

(d) Cash and cash equivalents

For the purpose of the statement of cash flows, cash and cash equivalents include cash on hand and at call deposits with banks.

(e) Investments

Investments are carried at fair value. Changes in fair value will be held in an equity reserve until the asset is disposed, at which time the changes in fair value will be brought to account through the Statement of Comprehensive Income.

(f) Revenue

Interest and dividends are recognised when received.
Grants, donations and distributions income are recognised when received.

(g) Goods and Services Tax (GST)

All revenue, expenses and assets are recognised net of the amount of goods and services tax (GST), except where the amount of GST incurred is not recoverable from the Australian Tax Office. In these circumstances the GST is recognised as part of the cost of acquisition of the asset or as part of an item of the expense. Receivables and payables in the statement of financial position are shown inclusive of GST.

(h) Financial instruments

Recognition and initial measurement

Financial instruments, incorporating financial assets and financial liabilities, are recognised when the entity becomes a party to the contractual provisions of the instrument.

Financial instruments are initially measured at fair value plus transactions costs where the instrument is not classified as at fair value through profit or loss. Financial instruments are classified and measured as set out below.

Classification and subsequent measurement

(i) Loans and receivables

Loans and receivables are non-derivative financial assets with fixed or determinable payments that are not quoted in an active market and are subsequently measured at amortised cost using the effective interest rate method.

(ii) Held-to-maturity investments

Held-to-maturity investments are non-derivative financial assets that have fixed maturities and fixed or determinable payments, and it is the entity's intention to hold these investments to maturity. They are subsequently measured at amortised cost using the effective interest rate method.

(iii) Available-for-sale financial assets

Available-for-sale financial assets are non-derivative financial assets that are either designated as such or that are not classified in any of the other categories. They comprise investments in the equity of other entities where there is neither a fixed maturity nor fixed or determinable payments.

(iv) Financial liabilities

Non-derivative financial liabilities (excluding financial guarantees) are subsequently measured at amortised cost using the effective interest rate method.

Fair value

Fair value is determined based on current bid prices for all quoted investments. Valuation techniques are applied to determine the fair value for all unlisted securities, including recent arm's length transactions, reference to similar instruments and option pricing models.

Impairment

At each reporting date, the entity assesses whether there is objective evidence that a financial instrument has been impaired. In the case of available-for-sale financial instruments, a prolonged decline in the value of the instrument is considered to determine whether an impairment has arisen. Impairment losses are recognised in the statement of comprehensive income.

(i) Impairment of assets

At each reporting date, the entity reviews the carrying values of its assets to determine whether there is any indication that those assets have been impaired. If such an indication exists, the recoverable amount of the asset, being the higher of the asset's fair value less costs to sell and value in use, is compared to the asset's carrying value. Any excess of the asset's carrying value over its recoverable amount is expensed to the statement of comprehensive income.

Where it is not possible to estimate the recoverable amount of an individual asset, the entity estimates the recoverable amount of the cash-generating unit to which the asset belongs.

2 Members' guarantee

If the company is wound up the Memorandum of Association states that each member is required to contribute a maximum of $2.00 each towards meeting any outstanding obligations of the company.

	2010 $	2009 $
3 Cash and Cash Equivalents		
General Account	1,033,641	504,716
Donations Account	18,775	36,667
D.W. Research Fund Account	327,513	215,651
	1,379,928	757,034
4 Receivables		
Sundry Debtors	97,333	237,225
	97,333	237,225
5 Investments		
Shares in listed corporations and other securities	6,622,732	5,810,046
Total available-for-sale financial assets	6,622,732	5,810,046
Held-to-maturity investments		
Bank Bills – at cost	840,000	1,765,000
Total held-to-maturity investments	840,000	1,765,000
Total Investments	7,462,732	7,575,046

	2010 $	2009 $
6 Plant and Equipment		
Office equipment – at cost	2,288	2,288
Less: Accumulated depreciation	(2,048)	(1,904)
	240	384
Reconciliation		
Reconciliation of the carrying amount of plant and equipment at the beginning and end of the current and previous financial year:		
Carrying amount at beginning of year	384	614
Less: Depreciation expense	(144)	(230)
Carrying amount at end of year	240	384
7 Payables		
Creditors and Accruals	20,562	20,787
Grants Payable	445,956	359,074
Director of Research – Victoria (refer note 15)	144,000	164,000
	610,518	543,861
8 Provisions		
Employee Benefits	13,307	14,147
9 Research Capital Fund		
General		
Balance 1 July 2009	586,990	579,547
Allocation to Capital:		
– 5% Surplus & Imputation Credits & Other Legacies	9,455	7,443
Balance 30 June 2010	596,445	586,990
Anselmi Estate		
Balance 1 July 2008	429,933	290,979
Distribution during year	–	138,954
Transfer during year	(138,954)	–
Balance 30 June 2010	290,979	429,933
Ivy May Stephenson Estate		
Balance 1 July 2009	36,833	30,376
Distribution during the year	–	6,457
Transfer during year	(6,457)	–
Balance 30 June 2010	30,376	36,833
Total	917,800	1,053,756
10 Settled Funds		
D.W. Research Funds	200,000	200,000
Esme Anderson	124,326	124,326
G.J. Williams	25,500	25,500
B. Mitchell	26,023	26,023
Dame Ida Mann (Est. 31/03/84)	56,707	56,707
Ronald and Lois Lowe	40,000	40,000
	472,556	472,556

	2010 $	2009 $
11 Financial Assets Reserve		
Balance 1 July 2009	150,684	607,788
Revaluation increment/(decrement)	173,250	(457,104)
Balance 30 June 2010	323,934	150,684

Financial assets reserve records unrealised gains on revaluation of financial assets to fair value.

12 Capitalised Profit on Re-arrangement of Investments, Capital Distribution and Special Dividends

	Balance 01/07/2009	Allocation of Realised Profit/(Loss) on Re-arrangement of Investments, Capital Distributions and Special Dividends	Balance 30/06/2010
	$	$	$
Research Fund:			
General	112,444	5,536	117,980
Anselmi Estate	40,279	1,982	42,261
Ivy May Stephenson	100	6	106
D.W. Research Funds	4,548,741	135,131	4,683,872
Esme Anderson	793,506	38,671	832,177
G.J. Williams	136,107	6,696	142,803
B. Mitchell	134,171	6,696	140,867
Dame Ida Mann	189,688	9,404	199,092
Ronald & Lois Lowe	118,276	6,245	124,521
	6,073,312	210,367	6,283,678

	Note	2010 $	2009 $
13 Accumulated funds			
(a) Administration			
Accumulated Deficits – 1 July 2009		–	–
Total Comprehensive Income		(7,067)	(13,448)
Total available for appropriation		(7,067)	(13,448)
Aggregate of amounts transferred from Administration	13(b)	7,067	13,448
Accumulated Deficits – 30 June 2010		–	–
(b) Trust Fund			
Retained Income – 1 July 2009		261,373	332,879
Total Comprehensive Income		(71,822)	94,796
Total available for appropriation		189,551	427,675
Aggregate of amounts transferred to General/Capital Funds:			
Administration	13(a)	(7,067)	(13,448)
Research Trust		135,956	(152,854)
Retained Income – 30 June 2010		318,440	261,373

	2010 $	2009 $
14 Grants Allocated/Made During the Year		
Grants allocated in prior years written back		(59,410)
Prof P McCluskey		70,000
Prof S Graham, Prof A Avolio and Dr M Butlin		50,000
Dr K Valter-Kocsi, D J G Wong, Prof J M Provis and Dr M Madigan		41,450
Dr R Jamieson and Dr J Grigg		38,000
Dr L Robman and Prof R Guymer		67,500
A/Prof K A Trounce and Prof J Crowston		42,358
A/Prof G Gole and Dr N Barnett		48,320
Dr S Sharma, A/Prof J Craig and A/Prof N Voelcker		50,000
Dr J Wood & Dr G Chidlow		50,000
Dr W Shen		49,423
Dr A Kwan, A/Prof T Chirila and Dr D Harkin		25,000
A/Prof R Casson and Prof P Blumbergs		69,030
Dr A Hewitt		49,540
Prof K A Williams and Prof D J Coster	50,000	
Dr S Wickremasinghe and Prof R Guymer	43,550	
Prof M Gillies	49,960	
A/Prof J Craig and Dr K Laurie	49,200	
Dr K Burdon and Dr R Mills	49,650	
Dr S Watson, A/Prof L Foster and Dr M Sarris	49,950	
Dr P Sanfilippo and Prof D Mackey	28,700	
Dr L Robman	46,600	
Prof P McCluskey, Dr M Madigan Dr R M Conway, Prof N Rao	49,900	
Dr L Lim	48,100	
Ms F O'Hare	45,791	
Dr M Schache	49,905	
Dr J S Gilhotra and Dr M Dhanapala	35,435	
	596,741	591,211
Deduct Contribution from:		
RANZCO	40,000	40,000
RANZCO Eye Foundation	75,000	75,000
Renensson Bequest	25,000	25,000
Glaucoma Foundation	45,791	50,000
Vision Australia	–	25,000
	185,791	215,000
	410,950	376,211

	2010 $	2009 $
15 Funds Allocated to Director of Ophthalmic Research – Victoria		
Balance as at 1 July 2009	164,000	193,000
Interest for the year	1,024	4,748
Allocation for year	144,000	164,000
	309,024	361,748
Payment made to Director of Research	165,024	197,748
Balance as at 30 June 2010	144,000	164,000
16 Auditors Remuneration		
Auditing accounts	4,950	4,950
Other services	–	–
	4,950	4,950
17 Reconciliation of Net Cash Provided by Operating Activities to Results for year		
Net Surplus/(Deficit)		
– Trust Fund	311,795	(735,654)
– Administration	(7,067)	(13,448)
	304,728	749,102
Depreciation	144	230
Provision for Employee Benefits	(840)	2,020
(Increase)Decrease in Receivables	139,892	(93,947)
Increase/(Decrease) in Creditors and Accrued Expenses	(225)	1,144
Increase/(Decrease) in Grants Payable	86,882	(71,435)
Increase/(Decrease) in allocation to Director of Research – Victoria	(20,000)	(29,000)
Valuation (Gains)/Losses on avaioaboe-for-sale financal assets	(173,250)	457,104
(Profit)/Loss on Rearrangement of Investments	(210,367)	373,346
Net Cash Provided by/(Used In) Operating Activities	126,964	(109,640)

18 Disclosures on Directors and other Key Management Personnel

Directors

The following directors received grants during the year. These are detailed at note 14.

Professor Mark Gillies
Dr Richard Mills
Dr Stephanie Watson
Professor David Mackey
Dr R M Conway
Professor Peter McCluskey

The names of the directors who have held office during the financial year are:

A/Prof Mark D Daniell, Melbourne (Chairman)
Professor Mark Gillies, Sydney (Vice Chairman)
Professor Stuart L Graham, Sydney (Honorary Secretary)
A/Prof Robert Casson, Adelaide (Honorary Treasurer)
Dr R Max Conway, Sydney
Professor J Crowston, Melbourne
Dr Wilson Heriot, Melbourne
Dr Anthony Kwan, Brisbane
Professor David Mackey, Perth
Professor Peter J McCluskey, Sydney
Dr Richard Mills, Adelaide
Dr Richard Stawell, Melbourne
Dr Andrea Vincent, New Zealand
Dr Stephanie Watson, Sydney
Professor Tien Wong, Melbourne

Key Management Personnel

Other Key Management Personnel include Executive Officer, Anne Dunn Snape.

Key management personnel are those persons having authority and responsibility for planning, directing and controlling the activities of the entity, directly or indirectly, including any director (whether executive or otherwise) of that entity. Control is the power to govern the financial and operating policies of an entity so as to obtain benefits from its activities.

Key Management Personnel Compensation

Key Management Personnel has been taken to comprise the directors and one member of the executive management responsible for the day-to-day financial and operational management of the entity.

	2010 $	2009 $
(a) Short-term employee benefits	57,708	55,386
(b) Post-employment benefits	(840)	2,020
(c) Other long-term benefits	–	–
(d) Termination benefits	–	–
(e) Share-based payment	–	–
	56,868	57,406

19 Financial Instruments

(a) Financial Risk Management Policies

The entity's financial instruments consist mainly of deposits with banks, local money market instruments, short-term investments, accounts receivable and payable.

The entity does not have any derivative instruments at 30 June 2010.

(i) Treasury Risk Management

An investment committee consisting of Board members of the entity meet on a regular basis to analyse financial risk exposure and to evaluate treasury management strategies in the context of the most recent economic conditions and forecasts.

The committee's overall risk management strategy seeks to assist the entity in meeting its financial targets, whilst minimising potential adverse effects on financial performance.

Risk management policies are approved and reviewed by the Board on a regular basis. These include credit risk policies and future cash flow requirements.

(ii) Financial Exposures and Management Risk

The main risks the entity is exposed to through its financial instruments are interest rate risk, liquidity risk and credit risk.

Interest rate risk
Interest rate risk is managed with a mixture of fixed and floating rate debt.

Foreign currency risk
The entity is not exposed to fluctuations in foreign currencies.

Liquidity risk
The entity manages liquidity risk by monitoring forecast cash flows.

Credit risk
The maximum exposure to credit risk, excluding the value of any collateral or other security, at balance date to recognised financial assets, is the carrying amount, net of any provisions for impairment of those assets, as disclosed in the statement of financial position and notes to the financial statements.

The entity does not have any material credit risk exposure to any single receivable or group of receivables under financial instruments entered into by the entity.

Price risk
The group is not exposed to any material commodity price risk.

19 Financial Instruments (cont)

(b) Financial Instrument Composition and Maturity Analysis

The entity's exposure to interest rate risk, which is the risk that a financial instrument's value will fluctuate as a result of changes in market interest rates and the effective weighted average interest rates on those financial assets and financial liabilities, is as follows:

	Weighted Average Effective Interest Rate		Floating Interest		Fixed Interest Rate Maturing Within 1 year		Fixed Interest Rate Maturing 1 to 5 years		Non Interest Bearing		Total Carrying Amount Per statement of financial position	
	2010 %	2009 %	2010 $	2009 $	2010 $	2009 $	2010 $	2009 $	2010 $	2009 $	2010 $	2009 $
Financial Assets												
Cash and Cash Equivalents	4.25	2.45	1,379,928	757,034	–	–	–	–	–	–	1,379,928	757,034
Listed Investments												
Shares	N/A	N/A	–	–	–	–	–	–	6,622,732	5,810,046	6,622,732	5,810,046
Bank Bills	5.50	3.20	–	–	840,000	1,765,000	–	–	–	–	840,000	1,765,000
Receivables	–	–	–	–	–	–	–	–	97,333	237,225	97,333	237,225
Total Financial Assets			1,379,928	757,034	840,000	1,765,000	–	–	6,720,065	6,047,271	8,939,993	8,569,305
Financial Liabilities												
Payables	–	–	–	–	–	–	–	–	610,518	543,861	610,518	543,861
Total Financial Liabilities			–	–	–	–	–	–	610,518	643,152	610,518	543,861
Net Financial Assets			1,379,928	757,034	840,000	1,765,000	–	–	7,330,583	5,503,410	9,550,511	8,025,444

19 Financial Instruments (cont)

(c) Net Fair Values

The net fair values of listed investments have been valued at the quoted market bid price at balance date. For other assets and other liabilities the net fair value approximates their carrying value. No financial assets and financial liabilities are readily traded on organised markets in standardised form other than listed investments.

The aggregate net fair values and carrying amounts of financial assets and financial liabilities are disclosed in the statement of financial position and in the notes to and forming part of the financial statements.

(d) Sensitivity Analysis

Interest Rate Risk

The entity has performed a sensitivity analysis relating to its exposure to interest rate risk at balance date. This sensitivity analysis demonstrates the effect on the current year results and equity which could result from a change in this risk.

Interest Rate Sensitivity Analysis

At 30 June 2010, the effect on profit and equity as a result of changes in the interest rate, with all other variables remaining constant, would be as follows:

	Carrying amount	Interest rate risk			
	$	–1% Profit	+1% Profit	–1% Equity	+1% Equity
2010 Financial Assets					
Cash and Cash Equivalents	1.379.928	(137,993)	137,993	(137,993)	137,993
2009 Financial Assets					
Cash and Cash Equivalents	757,034	(7,570)	7,570	(7,570)	7,570

DIRECTORS' DECLARATION

The Directors of the company declare that:

1. The financial statements and notes as set out on pages 39–54:
   (a) comply with Accounting Standards and Corporations Act 2001; and
   (b) give a true and fair view of the financial position as at 30 June 2010 and performance for the year ended on that date of the company.

2. In the directors' opinion there are reasonable grounds to believe that the company will be able to pay its debts as and when they become due and payable.

The declaration is made in accordance with a resolution of the Board of Directors.

On behalf of the Board.

Assoc/Prof M Daniell
Director

Assoc/Prof R Casson
Director

Sydney, this 11th day of September, 2010

OMW

Orr, Martin & Waters
CHARTERED ACCOUNTANTS

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Balwyn Vic 3103

Tel: 9836 8222
Fax: 9836 8331
ABN 90 040 794 950

Partners:

John E Volders
Larry R Gilmour
Grant W Petering

Independent Audit Report to the Members of The Ophthalmic Research Institute of Australia

(A COMPANY LIMITED BY GUARANTEE) ACN 008 393 146

Report on the Financial Report

We have audited the accompanying financial report of The Ophthalmic Research Institute of Australia (the company), which comprises the statement of financial position as at 30 June 2010, and the trust fund statement of comprehensive income, administration statement of comprehensive income, statement of changes in equity and statement of cash flow for the year ended on that date, a summary of significant accounting policies, other explanatory notes and the directors' declaration.

Directors' Responsibility for the Financial Report

The directors of the company are responsible for the preparation and fair presentation of the financial statements in accordance with Australian Accounting Standards (including the Australian Accounting Interpretations) and the *Corporations Act 2001*. This responsibility includes designing, implementing and maintaining internal controls relevant to the preparation and fair presentation of the financial statements that are free from material misstatement, whether due to fraud or error; selecting and applying appropriate accounting policies; and making accounting estimates that are reasonable in the circumstances.

Auditor's Responsibility

Our responsibility is to express an opinion on the financial statements based on our audit. We conducted our audit in accordance with Australian Auditing Standards. These Auditing Standards require that we comply with relevant ethical requirements relating to audit engagements and plan and perform the audit to obtain reasonable assurance whether the financial statements are free from material misstatement.

An audit involves performing procedures to obtain audit evidence about the amounts and disclosures in the financial statements. The procedures selected depend on the auditor's judgment, including the assessment of the risks of material misstatement of the financial statements, whether due to fraud or error. In making those risk assessments, the auditor considers internal control relevant to the entity's preparation and fair presentation of the financial statements in order to design audit procedures that are appropriate in the circumstances, but not for the purpose of expressing an opinion on the effectiveness of the entity's internal control. An audit also includes evaluating the appropriateness of accounting policies used and the reasonableness of accounting estimates made by the directors, as well as evaluating the overall presentation of the financial statements.

We believe that the audit evidence we have obtained is sufficient and appropriate to provide a basis for our audit opinion.

Independence

In conducting our audit, we have complied with the independence requirements of the *Corporations Act 2001*. We confirm that the independence declaration required by the *Corporations Act 2001*, provided to the directors of The Ophthalmic Research Institute of Australia on 30th August 2010, would be in the same terms if provided to the directors as at the date of this auditor's report.

Auditor's Opinion

In our opinion the financial statements present fairly, in all material aspects, the financial position of The Ophthalmic Research Institute of Australia as at 30 June 2010, and its financial performance and cash flows for the year then ended in accordance with the Corporations Act 2001 and the Australian Accounting Standards (including Australian Accounting Interpretations).

Orr, Martin & Waters
Chartered Accountants

L.R. Gilmour, Partner

Dated this 30th day of August 2010.

Auditor's Independence Declaration under Section 307C of the Corporations Act 2001

I declare that, to the best of my knowledge and belief, during the year ended 30 June 2010 there have been:

(i) no contraventions of the auditor's independence requirements as set out in the *Corporations Act 2001* in relation to the audit; and

(ii) no contraventions of any applicable code of professional conduct in relation to the audit.

Orr, Martin & Waters
Chartered Accountants

L.R. Gilmour, Partner

Dated this 30th day of August 2010.

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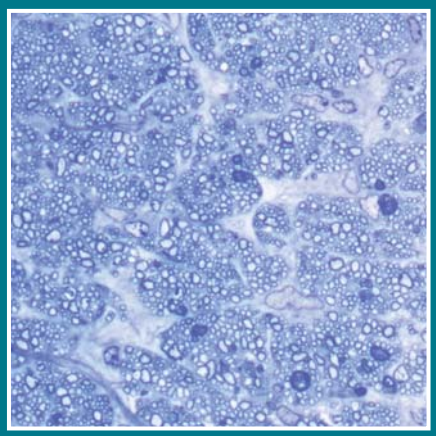

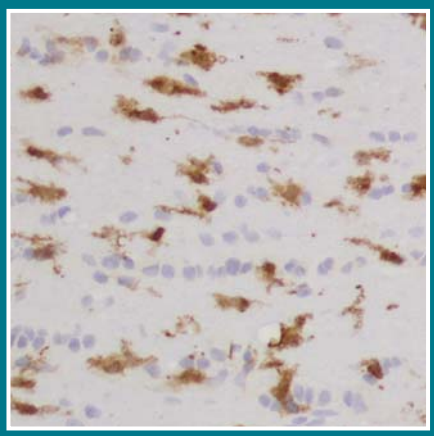

RESEARCH REPORTS AND FINANCIAL STATEMENTS