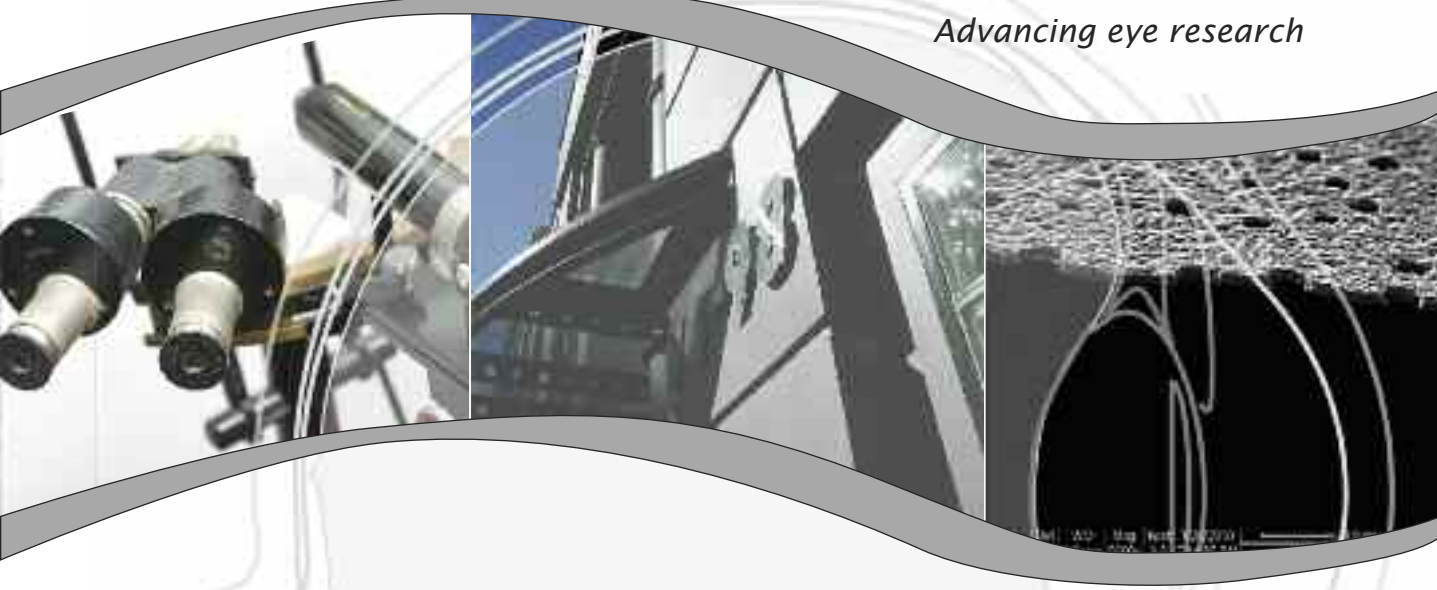
ORIA

Advancing eye research



Annual Report 2012

The Ophthalmic Research Institute of Australia

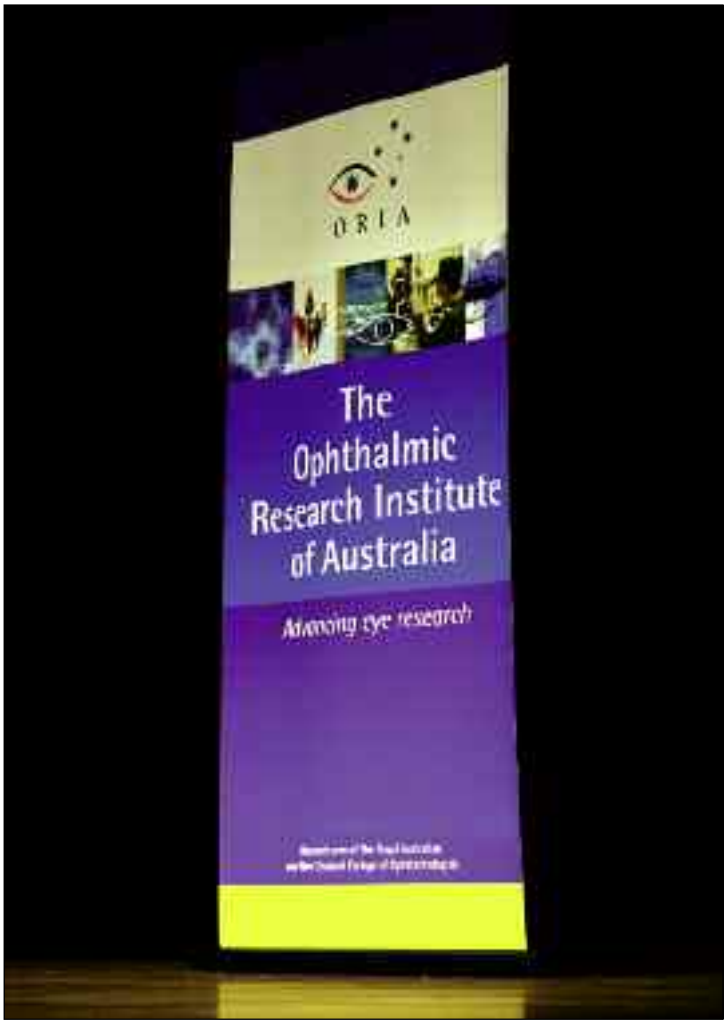
ABN 37 008 33 146

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 Sixtieth Annual General Meeting to be held at the Melbourne Convention and Exhibition Centre on Sunday 25 November 2012 at 8.30 am



THE BOARD	Professor Stuart Graham, Sydney (Chairman) Professor Mark Gillies, Sydney (Vice Chairman) Dr Richard Mills, Adelaide (Honorary Secretary) Dr W Heriot, Melbourne (Honorary Treasurer) Dr Fred Chen, Perth Dr Colin Clement, Sydney Professor J Crowston, Melbourne A/Prof Mark Daniell, Melbourne Dr Paul Healey, Sydney Dr Anthony Kwan, Brisbane Professor David Mackey, Perth Professor Peter J McCluskey, Sydney Dr John Males, Sydney Dr Andrea Vincent, New Zealand Dr Stephanie Watson, Sydney Professor Tien Wong, Melbourne
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HONORARY TREASURER	Dr Wilson Heriot 94-98 Chalmers Street, Surry Hills 2010
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AUDITORS	Orr Martin & Waters 461 Whitehorse Road, Balwyn 3103
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EXECUTIVE OFFICER	Ms Anne Dunn Snape, BA (Soc Sc& Pol Phil), (MU) PostGradC Ethics & Legal Studs

Chairman's report

ORIA marks 60 years of providing funding for research into eye health for the Australian community

The ORIA will mark its 60th Annual General Meeting on November 25th 2012 with the ORIA being formally registered on the thirteenth day of October 1953. The ORIA, since that time has provided millions of dollars from its virtual institute, towards eye health in the Australian community. We are indebted to all those who have supported and worked towards its goals of advancing eye research in Australia.

The ORIA's activities are co-ordinated and managed by the 16-member Board of the ORIA and Executive Officer, Anne Dunn Snape who celebrated 10 years in March with the organization. Using the income from its investments and donor organisations, the ORIA continued to contribute to funding for research projects throughout Australia during the financial year.

The ORIA's Research Advisory Committee is composed of leading research scientists and ophthal-mologists 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 the Committee are put forward to the Board of the ORIA who then indicate what funds are available for the forthcoming calendar year.

Project funding in 2012

During the year the ORIA's Research Advisory Committee considered 33 applications for project funding from Australian researchers, a significant increase from 19 assessed in 2004. It also assessed six 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.

This year $534,695 was distributed to fund 11 one year projects. The RANZCO Eye Foundation contributed $100,000 towards co-supporting three projects and Glaucoma Australia Inc. $72,005 to co-support three projects. We are most grateful to both organisations for their continuing support along with previous benefactors whose legacies are acknowledged through the naming of individual grants.

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 from January 2012 were:

ORIA/RANZCO Eye Foundation Grant
Dr Alex Hewitt & Dr Stuart Macgregor – *Methylation Profiles in Patients with Age-Related Macular Degeneration* **$46,270**

ORIA/Quinlivan & Glaucoma Australia Grant
Dr Glyn Chidlow – *Investigations into optic nerve injury in a rat model of glaucoma* **$49,960**

ORIA/Quinlivan & Glaucoma Australia Grant
A/Prof Jamie Craig & Dr David Dimasi – *Investigation of methylation status at the 9p21 glaucoma susceptibility locus between glaucoma cases and controls* **$49,250**

ORIA/RANZCO Eye Foundation Grant
Dr John Wood – *Axonal Regeneration in Experimental Glaucoma* **$49,725**

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.


Ms Patricia Poulos, ORIA's Grants Administrative Officer

Good governance and valuable outcomes

The ORIA is always mindful of auditing its research funding to assess how well its mission to advance eye research is being achieved. Each year, progress/final reports are provided from researchers funded during the previous year. A financial statement from each project/institution is also secured to ensure funds have been used in the manner previously indicated in the application. In 2011, two of our funded projects have received NH&MRC funding to around $3 million based on the research supported by the ORIA. A further two of the successful ORIA funded projects in 2011 await notification of NH&MRC funding.

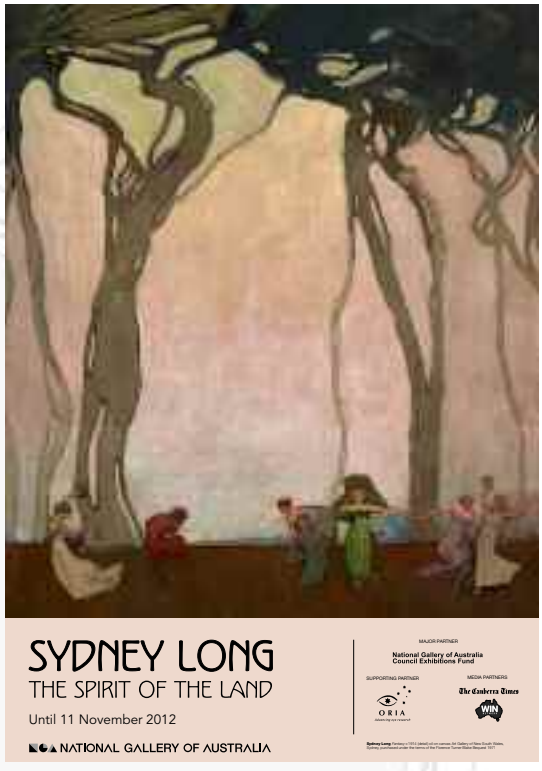
As well ...

During the year, the ORIA launched a new and contemporary website at www.oria.org.au. This has been designed with a view to the future and to be a more engaging interface with both scientists and the public.

The ORIA has continued its support of the Australasian Ophthalmic and Visual Sciences Meeting (AOVSM). The meeting ran concurrently last year at RANZCO in Canberra and it has been agreed to continue this format with this year's RANZCO meeting in Melbourne from the 26th to 28th November 2012. Program details can be accessed via the RANZCO Congress website.

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

During 2012 the ORIA has entered into a partnership with the National Gallery of Australia, Canberra for its Sydney Long's *The Spirit of the Land* exhibition which runs from 17 August to 11 November 2012 in Canberra. The ORIA was bequeathed the reproduction of copyright rights for Sydney Long's works. The partnership has enabled the ORIA's brand to be used on all national publicity in print, electronic and media. A leaflet under the title ***ORIA: Helping Australians to see more clearly***, is being distributed at the NGA during the course of the exhibition. We encourage all to visit and support the beautiful exhibition. http://nga.gov.au/Long/



During the year, the ORIA saw some changes to its Board. We welcomed Dr Fred Chen from Perth, Dr Paul Healey from Sydney and Dr Colin Clement from Sydney to the Board. A/Prof Mark Daniell stepped down as Chair and Prof Robert Casson as Treasurer. New office bearers are Chair, Prof Stuart Graham from Sydney, Hon Treasurer, Dr Wilson Heriot from Melbourne and Hon Secretary, Dr Richard Mills from Adelaide.

***Prof Stuart Graham**, Chair, ORIA*

Anne Dunn Snape, Stuart Graham, Bob Casson and Mark Daniell at the ORIA's AGM.

ORIA grants awarded in 2012

ORIA/RANZCO Eye Foundation Grant
Dr Alex Hewitt & Dr Stuart Macgregor
Methylation Profiles in Patients with Age-Related Macular Degeneration **$46,270**

ORIA/Quinlivan & Glaucoma Australia Grant
Dr Glyn Chidlow
Investigations into optic nerve injury in a rat model of glaucoma **$49,960**

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A/Prof Jamie Craig & Dr David Dimasi
Investigation of methylation status at the 9p21 glaucoma susceptibility locus between glaucoma cases and controls **$49,250**

ORIA/RANZCO Eye Foundation Grant
Dr John Wood
Axonal Regeneration in Experimental Glaucoma **$49,725**

ORIA/Esme Anderson Grant
Dr Shiwani Sharma, Dr Kathryn Burdon & Prof Jozef Gécz
Finding congenital cataract causing genetic defects using the latest methodology **$50,000**

ORIA/RANZCO Eye Foundation Grant
Dr Rohan Essex, Dr Willie Campbell, Dr Alex Hunyor Jr & Dr Paul Connell
A prospective cohort study evaluating the predictors of outcome in macular hole surgery **$48,755**

ORIA/Quinlivan & Glaucoma Australia Grant
A/Prof Ian Trounce
Does the loss of the 'good' Alzheimer's protein in the older eyes contribute to glaucoma? **$44,800**

ORIA/G J Williams Grant
Prof Ian McAllister, A/Prof LRS Vijayasekaran, Prof Degli-Esposti & A/Prof Yu
Intravitreal Tenecteplase (metalyse) (TNK) as an acute treatment for blockages in retinal veins (RVOs) **$49,995**

ORIA/Renensson Bequest Grant
Dr Weiyong Shen & Dr Ling Zhu
Target Muller cell dysfunction for treatment of retinal diseases **$50,000**

ORIA New Investigator Grant
Dr Hannah Forward, Dr Charlotte McKnight & Dr Alexander Tan
Raine Eye Health Study – Ocular Biometry and Ultraviolet Exposure **$50,000**

ORIA New Investigator Grant
Dr Jelena Kezic
Macrophages in the Ageing Eye **$45,940**

TOTAL: $534,695

THANKS

With many thanks for donations to:

Glaucoma Australia Inc; The RANZCO Eye Foundation; Dr Garry Brian; Mr Frank Curphey

The Institute would like to thank our external referees who kindly gave advice which helped with the allocation of the 2012 grants.

Alex Harper, Melbourne
Alex Hewitt, Melbourne
Alex Tan, Perth
Alexander Dobrovic, Melbourne
Algis Vingrys, Melbourne
Andrew Chang, Sydney
Andrew Thompson, New Zealand
Antonio Giubilato, Perth
Colin Willoughby, UK
Con Petsoglou, UK
David Pow, Brisbane
Dipka Patel, New Zealand
Damien Harkin, Brisbane
Ecosse Lamoureux, Melbourne
Elfride de Baere, Belgium
Fred Chen, Perth
Geoffrey Crawford, Perth
Glyn.Chidlow, Adelaide
Hannah Forward, Perth
Helen Brereton, Adelaide
Ian Constable, Perth
Ian McAllister, Perth
Ian Trounce, Melbourne
Ivan Goldberg, Sydney
Jan Kahn, Perth
Jelena Kezic, Melbourne
Jennifer Craig, New Zealand
Jill Keeffe, Melbourne
John Foster, Sydney
John McAvoy, Sydney
John Phillips, New Zealand
John Wood, Adelaide
Jonathan Stone, Canberra
Julie Lim, New Zealand
Kathryn Burdon, Adelaide
Keith Martin, Cambridge UK
Keren Abberton, Melbourne
Keryn Williams, Adelaide
Leslie Burnett, Sydney
Lyndell Lim, Melbourne
Mark Walland, Melbourne
Michelle Madigan, Sydney
Monica Acosta, New Zealand
Nick Martin, Brisbane
Patrick Humbert, Melbourne
Penny McKelvie, Melbourne
Peter Van Wijngaarden, Melbourne and Cambridge
Robyn Guymer, Melbourne
Robyn Jamieson, Sydney
Roger Truscott, Sydney
Sam Fraser-Bell, Sydney
Shiwani Sharma, Adelaide
Sonja Klebe, Adelaide
Stewart Lake, Adelaide
Ted Maddess, Canberra
Tien Wong, Singapore
Traian Chirila, Brisbane
Trevor Lamb, Canberra
Vicki Chrysostomou, Melbourne
Weiyong Shen, Sydney
Wilson Heriot, Melbourne

The ORIA is always grateful to its peer review panel whose assessments of applications for funding are invaluable. The reviewer we deemed as providing the "most useful" review for funding in 2012 was Dr Peter Van Wijngaarden. Peter is currently studying at Cambridge University, UK.

Prof Peter McCluskey, Marian McCluskey, Anne Dunn Snape and Danielle Symons at the opening of the Sydney Long 'The Spirit of the Land' Exhibition at the National Gallery of Australia, Canberra in August.

Progress reports on research supported by ORIA Institute grants 2011

ORIA/Esme Anderson Grant

What role does immunity play in Age-related Macular Degeneration (AMD)

A/Prof Paul Baird

Aims

Aim 1. To investigate association of HLA class II genes with risk of AMD
Aim 2. To investigate association of HLA class II genes with known AMD genes

Background

Age-related Macular Degeneration (AMD) is a progressive neurodegenerative disease detected in 1 in 7 (14%) of Australians over the age of 50, but 1 in 4 by the age of 90. It accounts for nearly half of all legal blindness in elderly Australians (24,000 individuals) and is the leading cause of blindness in the developed world. There is overwhelming evidence to indicate that genetics plays a key role in AMD. Since 2005 and the discovery of the complement factor H (*CFH*) gene as being associated with AMD, variants in at least 11 other genetic loci have also been reported as associated with AMD with the majority of these being immune related.

Immune function in humans is dependent on the human leukocyte antigen (HLA) system, synonymous with the human major histocompatibility complex (MHC) – a region containing more than 200 genes at chromosome position 6p21.3. This region is critical for our immune response through presentation of processed antigenic peptides to both CD4 helper and CD8 cytotoxic T lymphocytes as well as recognition of self. The HLA region is composed of the class I, II and III regions.Given the inflammatory/immune component in AMD and association of the C2/BF AMD associated genes in the class III region, we wished to assess if other HLA regions involving class I and class II antigens may also play a role in susceptibility to AMD.

Methods

AMD case and control samples from our patient repository were genotyped for multiple genetic variants in AMD risk associated genes including *CFH, C3, CFHR1-5, C2/BF, HTRA1/ARMS2* and *APOE* genes using a MassArray platform (SEQUENOM). Amplification of alleles of HLA Class I and II antigens was undertaken using an HLA Sequence Based Typing (SBT) procedure. Statistical analysis (undertaken using SPSS) comparing cases with controls adjusting for age, sex, and smoking status allowed estimation of odds ratios (OR) and p values.

Results

Aim 1. To investigate association of HLA class II genes with risk of AMD disease

HLA-DRB1 and *HLA-DQB1* alleles were genotyped in 250 advanced AMD cases and 245 controls. A total of 13 *HLA-DRB1* alleles were identified. Significant association following Bonferroni correction (corrected p=0.004 (0.05/13)) was evident for *HLA-DRB1*15* as a protective allele (OR=0.59, 95% confidence intervals (95% CI) 0.41-0.81), p=0.003 compared to all other alleles combined. Whereas 2 other alleles were identified with significant risk including *DRB1*03* and *DRB1*04*, with the most significant being *DRB1*04* (OR=2.3, 95% CI 1.4-3.6), p<0.0001. Among *HLA-DQB1* alleles, a total of 4 alleles were significantly associated with AMD following Bonferroni correction (corrected p=0.01 (0.05/5). The *HLA-DQB1*6.02*

allele was the most protective (OR=0.55, 95% CI 0.41, 0.74), p<0.0001 whereas the alleles *HLA-DQB1*2*, *HLA-DQB1*3* and *HLA-DQB1*5* were found to be risk with the highest risk being for *HLA-DQB1*2*, (OR=1.9, 95% CI 1.3, 2.8), p<0.0001 and *HLA-DQB1*3* (OR=1.9, 95% CI 1.4, 2.7), p<0.0001 respectively compared to *HLA-DQB1*6.02*.

Aim 2. To investigate association of HLA class II genes with known AMD genes

We assessed the association of the *HLA-DRB1*15.02* allele and common genetic variants in known AMD genes: the SNP rs10490924 in the *LOC387715 (ARMS2)* gene on chromosome 10, and the rs1410996 and Y402H SNPs in the CFH gene on chromosome 1. Risk of AMD decreased from an OR of 6.6 for the T risk allele of the SNP rs10490924 in the *LOC387715* gene to an OR of 2.0 when the *HLA-DRB1*15.02* allele was also present. In the case of the C allele of Y402H of *CFH*, risk drops from OR 4.7 to OR 2.3 whereas for the C risk allele of the SNP rs1410996 of the *CFH* gene, the risk drops from an OR of 15.5 to an OR of 5.2 in the presence of the *HLA-DRB1*15* allele. Thus the *HLA-15* allele appears to at least halve the risk of AMD conferred by known AMD risk genes.

These findings point to a pivotal role of HLA alleles in AMD with different alleles providing risk and protective associations with advanced AMD. In addition, these alleles influence the risk of other already known AMD genes through up to a halving of disease risk. Thus the importance of these findings cannot be understated if we are to better target groups for future clinical trials for a personalised medicine approach.

Presentations

Richardson AJ, Disanto G, Ebers GC, Morrison K, Guymer RH, Baird PN. Investigating association of HLA Class II genes and Age-related Macular Degeneration (AMD). Poster Presentation. 8th Australasian Human Gene Mapping Conference, April 2011, Hobart, Tasmania.

ORIA New Investigator Grant

Bioengineered Corneal Endothelium

Dr Karl Brown, A/Prof Mark Daniell, Dr Keren Abberton & Dr Berkay Ozcelik

The corneal endothelium is critical for maintaining the fluid balance of the corneal stroma. If the fluid efflux provided by the corneal endothelium drops below a critical threshold, then stromal oedema results in corneal opacity and painful bullous. Loss of corneal endothelial cells (CECs) can have a number of aetiologies including: aging, accidental damage during surgery, or Fuch's dystrophy. The rate of adult human corneal endothelial cell proliferation is too low to replace losses. Limited monolayer repair is mostly involves enlargement and migration of cells. If this limited capacity to repair is exceeded, surgical intervention is required.

The current treatments for human CEC (HCEC) loss are transplantation, either a full thickness keratoplasty or a component transplantation such as Descemet's Stripping Endothelial Keratoplasty (DSEK). In DSEK, donor endothelium is able to restore transparency by abating oedema in recipient corneal stroma. These treatments have problems including the risk of rejection, the need for immunosupression and associated side effects, the possibility of graft-host transmission of disease, and limited donor tissue supply. A bioengineered corneal endothelium could potentially eliminate these problems.

Key findings

Our approach is to culture corneal endothelial cells on a thin (50 μm) hydrogel film suitable for implantation. Several formulations of hydrogel films were successfully fabricated using a new method of cross-linking which demonstrated suitable physical properties (including tensile strength and transparency)

and were capable of supporting attachment and proliferation of CECs in culture. Formulations included fully synthetic films and hybrid films incorporating chitosan. Results for Chitosan-PEG hybrid hydrogel films (HHFs) are given here as a typical example. Other films produced similar characteristics. Tensile testing of HHFs demonstrated significantly enhanced mechanical properties superior to other corneal tissue engineering materials produced previously. Table I summarises the range of tensile properties for the films we generated, which are comparable to those of the human cornea.

Material	US (MPa)	UE (%)	EM (MPa)
Chitosan Film	9.91 ± 1.55	64.5 ± 11.5	15.5 ± 2.72
HHF 10% PEGDGE	12.7 ± 2.86	70.3 ± 6.02	12.5 ± 1.80
HHF 30% PEGDGE	22.9 ± 1.53	103 ± 9.01	14.3 ± 5.10
HHF 50% PEGDGE	28.2 ± 3.34	101 ± 17.4	17.7 ± 6.60
HHF 70% PEGDGE	11.9 ± 3.68	81.3 ± 11.5	12.6 ± 0.81
HHF 100% PEGDGE	5.92 ± 2.08	37.9 ± 8.39	10.4 ± 3.43
Human Cornea	3.3 ± 0.19	60.0 ± 15.0	15.9 ± 1.96

Table I: Ranges of mechanical properties for the HHFs compared to that of the human cornea and unmodified chitosan film. US: Ultimate stress, Force needed to stretch the material to breaking point. UE: Ultimate elongation, the percentage increase in length prior to breaking. EM: Elastic Modulus, a measure of stiffness and flexibility.

Transmission of visible light through the HHFs was found to be greater than 95%, which makes them highly suitable for ophthalmic applications. *Ex vivo* ocular implantation via DSEK demonstrated the tensile integrity and robustness of the HHFs (data not shown). It is important for ophthalmic implants to be permeable to large and small biomolecules. The diffusivities for glucose and albumin through the HHFs were found to be ~1.0 x 10^{-6} cm^2/s and ~1.0 x 10^{-8} cm^2/s respectively. In comparison, the human cornea has diffusivities for glucose and albumin of ~2.6 x 10^{-6} cm^2/s and ~1.0 x 10^{-7} cm^2/s respectively. Therefore, HHFs have diffusivities for glucose and albumin comparable to that of the human cornea.

In vitro degradation studies in the presence of lysozyme and L-cysteine over an 8 week period demonstrated that the HHFs are biodegradable with a mass loss of greater than 50% in 8 weeks at 100X the concentration of lyszoyme present in the human aqueous humour. Degradation products of the HHFs were found to be non-toxic *in vitro* over a 72h study with 3T3 fibroblasts. An *in vitro* model of inflammation found no activation of ovine peripheral blood mononuclear cells in the presence of fully synthetic film or HHF (data not shown).

Naturally non-proliferative CECs from sheep and humans were successfully grown to confluence on a variety of surfaces that have the potential to be implanted using DSEK. Preliminary analysis of suspension cultures for (stem cell derived) sphere forming assay indicates that adult stems cells are present at approximately 1:2500 cells in the ovine corneal endothelium. We determined the cell density of ovine corneal endothelium to be 3150cell/mm^2 in 14-month-old sheep (similar to that of humans). Therefore, the average density of adult stem cells over the surface of the ovine cornea is 1 per 1.26mm^2. Ongoing studies will determine the optimal seeding density of corneal endothelial stem cells to obtain cultured corneal endothelium of clinically acceptable cell density on a selected synthetic hydrogel film.

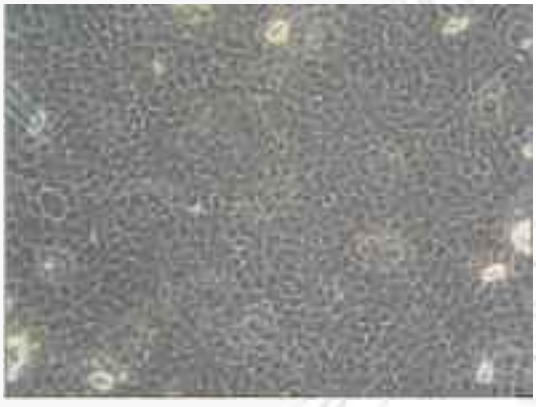


Figure 1. OCEC on fully synthetic hydrogel film. Day 20 of culture. Objective x20.

The bioengineered corneal endothelium resulting from the combination of a hydrogel film and cultured corneal endothelium is suitable for pre-clinical *in vivo* trials.

Unexpected limited availability of human and sheep tissues suitable as sources of CEC has delayed completion of some experiments. Consumables including antibodies funded by this grant remain and will be used to complete additional studies as tissue becomes available.

Publication in preparation

Chitosan-PEG hybrid hydrogel films for corneal tissue engineering. Berkay Ozcelik, Karl Brown, Anton Blencowe, Mark Daniell, Geoff Stevens, Greg Qiao.

Presentations at national and international conferences

A hydrogel film for corneal endothelium bioengineering. Karl D. Brown, *et al*. Asia ARVO 2011, Singapore.

Corneal tissue engineering. Karl D. Brown *et al*. 28th Australia & New Zealand Cornea Society Meeting 2011, Sydney, NSW.

Ultrathin, biodegradable and biocompatible hydrogel films for corneal endothelium regeneration. Berkay Ozcelik, et al. Australasian Polymer Symposium, 2011, Coffs Harbour, NSW, Australia.

Biodegradable and biocompatible hydrogel films for corneal tissue engineering. Berkay Ozcelik, *et al*. Asia-Oceania Top University League on Engineering (AOTULE) Conference, 2011, Beijing, China.

Stem cell transfer technology-from test tube to the eye. MD Daniell, K Brown and H Zhang. 29th Australia & New Zealand Cornea Society Meeting 2012, Auckland, New Zealand.

ORIA/WA Quinlivan/Glaucoma Australia Inc Grant

Genetic causes of childhood blindness in Sri Lanka and Cambodia

Dr Kathryn Burdon, A/Prof Jamie Craig, Dr James Muecke, Dr Adam Rudkin & Dr Jillian Nicholl

The goal of this project was to advance understanding of the genetic causes of childhood blindness in South East Asia, in particular Anterior Segment Dysgenesis and Primary Congenital Glaucoma in Sri Lanka and Cambodia. The study is based on data and samples collected as part of detailed surveys of the causes of blindness in children attending schools for the blind in these two countries. Children with multiple similarly affected family members were invited to provide a saliva sample for genetic studies. Samples were also collected from any available family members, particularly parents and siblings.

We have screened all children documented as have Primary Congenital Glaucoma (PCG) or buphthalmos for mutations in the *CYP1B1* gene, known to cause PCG. Twelve cases were sequenced. Homozygous mutations in CYP1B1 were present in four cases of PCG/buphthalmos. This included the missense mutation c.1169G>A which correlates with the protein change p.Arg390His (p.R390H) which was identified in two index cases from Cambodia. The *CYP1B1* missense mutation c.517G>A. was found in a third Cambodian case. A heterozygous mutation in the *CYP1B1* gene (c.850C>T) which correlated with the protein change p.Arg284Trp was sequenced in a Cambodian child with a dense vascualrised opacification of an enlarged left globe, and a right phythsical globe. This is an variant of *CYP1B1* which has not been described previously. A heterozygous change c.685G>A was found in a Sri Lankan case which was characterised by gross anterior segment disorganisation.

Similarly, we have screened the PAX6 gene for mutations in 25 cases from Cambodia, and 22 cases from Sri Lanka with either classic Aniridia or an anterior segment dysgenesis phenotypes reminiscent of aniridia. In the Sri Lankan cohort a single mutation was identified in a patient with iris aplasia, cataract and glaucoma – mutation G>T, 3rd base of start codon (p.M1R) which would likely result in a failure of protein translation. In the Cambodian cohort, a PAX6 mutation (c.1267dupT) was identified in one case of classic anirdia. The mutation was a C-terminal extension mutation. It is predicted to result in string of Lys attached to the end of the PAX6 protein (p.X423L run on), leading to translation into 3'UTR.

A major goal of the project was to identify novel genes for these phenotypes in this unique cohort. In particular, we undertook positional cloning of the causative gene in family CA2 from Cambodia, who

presented with a PCG/buphthalmos phenotype as well as corneal opacity and vascularisation. We have previously mapped the gene to a small region on chromosome 2 by homozygosity mapping in this recessive family. During the course of this project, we sequenced all annotated genes in this linkage region and identified only 1 homozygous, segregating coding variant. This was an Arg341X mutation in the *PXDN* gene. Through our collaborative networks we determined that collaborators had also identified mutations in this same gene in two families from Pakistan with a similar corneal phenotype. The discovery of this gene lead to a publication in the highly ranked and well regarded American Journal of Human Genetics. We have since received requests from International clinicians requesting investigation of this gene in their patients with similar phenotypes.

We have now sequenced the *PXDN* gene in all samples from Cambodia and Sri Lanka with phenotypes similar to either our Cambodian family or the Pakistani families, but have not identified any additional mutations. We have also screened the gene in our repository of PCG patients from Australia and similarly have not identified any mutations. We now believe that this gene is responsible for a primary corneal opacity/microphthalmia phenotype and the glaucoma observed in our Cambodian family is likely secondary to this. This finding is being prepared for publication.

We have further advanced our gene discovery projects in this cohort through the "whole-exome" sequencing of five Cambodian families with PCG or ASD. This has involved the sequencing of all known genes in the human genome in multiple family members through "Next Generation Sequencing". This approach is being used more and more widely and is most likely to be successful when the phenotype is recessive, as observed in all these families. A large number of novel variants were identified in each exome sequenced, but by analysis across family members and filtering of variants by their predicted effect on the protein, the number of mutations of interest was narrowed to those likely to be functional. In one family in particular we have identified a candidate gene for further investigation. It contains the only novel truncating variant that segregates in all samples sequenced so far. We are currently confirming the sequencing results and checking the mutation for segregation in additional family members. We have a number of controls from Cambodia which we will also screen. If this gene appears to be responsible for the phenotype in this family, we will screen the gene in additional cases with a similar phenotype from our repository of both South East Asian and Australian patients.

Publications resulting from this grant to date

Khan, K, A Rudkin, DA Parry, KP Burdon, M McKibbin, CV Logan, ZI Abdelhamed, JS Muecke, N Fernandez-Fuentes, KJ Laurie, M Shires, R Fogarty, IM Carr, JA Poulter, JE Morgan, MD Mohamed, H Jafri, Y Raashid, N Meng, H Piseth, C Toomes, RJ Casson, GR Taylor, M Hammerton, E Sheridan, CA Johnson, CF Inglehearn, JE Craig and M Ali, 2011. Homozygous Mutations in PXDN Cause Congenital Cataract, Corneal Opacity, and Developmental Glaucoma. *American journal of human genetics* 89(3): 464-473.

ORIA New Investigator/WA Quinlivan/Glaucoma Australia Grant

The impact of exercise on the response of aged retinal ganglion cells to injury

Dr Vicki Chrysostomou

Background and aims

The prevalence and incidence of glaucoma increases exponentially with age yet the pathophysiology underlying age as a risk factor is unclear. Mitochondrial dysfunction is seen with advancing age and has been shown to act causally in several neurodegenerative diseases. Mitochondrial abnormalities have recently been reported in patients with primary open-angle glaucoma. Given these observations, we hypothesise that age-

related mitochondrial dysfunction, and subsequent defects in cellular energy production, renders retinal ganglion cells susceptible to glaucomatous injury.

Exercise is known to improve mitochondrial function and can protect against a range of diseases. There is good evidence that exercise is neuroprotective; in the brain, physical activity protects neurons during normal ageing, following injury, and in neurodegenerative conditions. The role of exercise in eye health and its effect on retinal cell biology and function, however, is largely unstudied. The aim of the current study was to test if exercise can protect aged retinal ganglion cells from a pressure-induced injury.

Progress and results to date

1. Exercise has no overt effect on baseline retinal electrophysiology or resting IOP. Over the past year, we completed experiments in which 12 month old C57BL/6 mice underwent 6 weeks of forced daily exercise. Swimming was chosen as the mode of exercise because it can be closely regulated in duration and load, and causes minimal agitation to animals. Mice are natural swimmers and in accordance with previous reports, our mice adapted quickly and expertly to swimming protocols. We found that 6 weeks of exercise had no effect on ERG responses derived from the inner retina (Figure 1A). Further, our exercise regime did not alter resting IOP levels (Figure 1B).

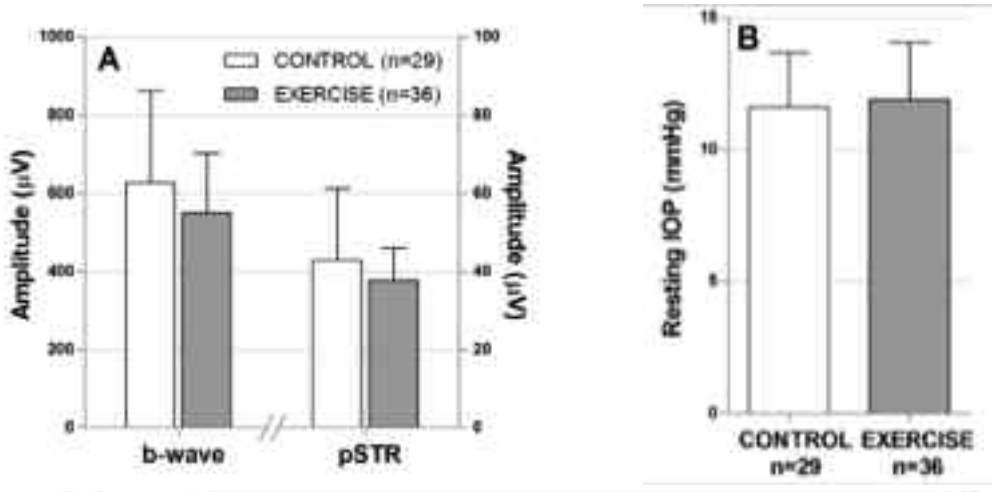


Figure 1. Mice subjected to daily exercise for 6 weeks showed no difference in (A) inner retinal ERG responses or (B) resting intra-ocular pressure (IOP) values compared to non-exercised control mice.

2. Exercise significantly improves functional recovery of the optic nerve following pressure injury. In two separate experiments conducted over the past year, 12-month-old exercised mice showed significantly less retinal ganglion cell dysfunction in response to pressure injury (acute elevation of IOP) compared to age-matched control mice (Figure 2). When results from the two experiments are pooled, we show that the protective effect of exercise is highly significant (P=0.001). To the best of our knowledge these data provide the first evidence that exercise can protect retinal cells from an experimental injury.

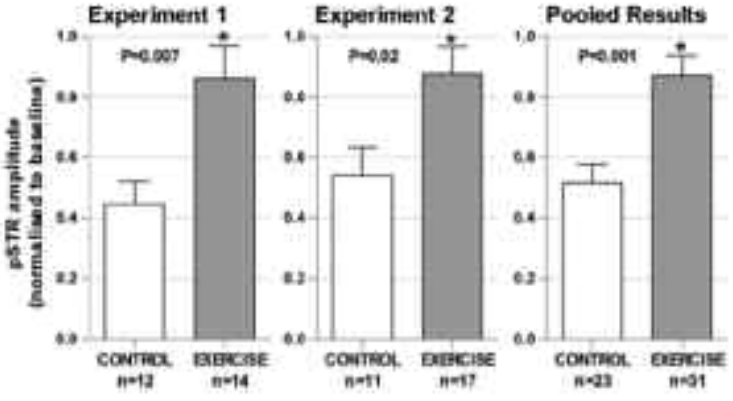


Figure 2. Exercise significantly improved functional recovery (pSTR amplitude) one week after IOP elevation.

3. Exercise reduces oxidative stress following pressure injury. In addition to reducing functional loss, exercise ameliorated injury-induced biochemical changes in the retina. Compared to IOP-challenged control retinas, retinas from exercised animals showed (i) significantly lower mRNA and protein expression levels of the stress response protein GFAP (Figure 3), (ii) significantly higher mRNA levels of the RGC-specific protein Thy-1, and (iii) reduced overall numbers of activated inflammatory (Iba1+) cells.

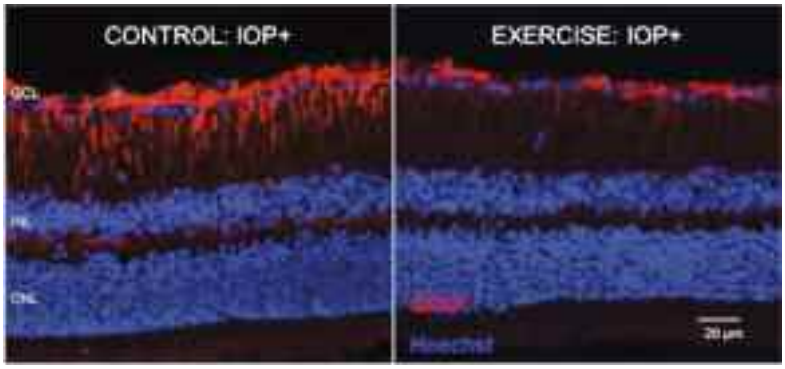


Figure 3. Exercise reduced retinal GFAP expression following IOP elevation.

Summary and future directions

Our data show that exercise significantly improves functional recovery of aged retinal ganglion cells following pressure injury. This protection is accompanied by signs of reduced oxidative stress. To our knowledge, these data provide the first evidence that exercise can protect the retina from injury. The encouraging results from this study have served as pilot data for an NHMRC Project Grant submitted in 2012, in which we propose to investigate the mechanisms underlying exercise-mediated protection of retinal cells.

ORIA/RANZCO Eye Foundation Grant

Investigation of the Role of G-CSF in uveitis

Dr Gabriel Goldberg, Dr Lyndell Lim & Dr Ian Wicks

Granulocyte colony stimulating factor (G CSF) is a major regulator of neutrophil production, function and survival. G-CSF binds the G-CSF receptor (G-CSF-R) which is primarily expressed by mature neutrophils but also macrophages, monocytes and myeloid progenitors in the bone marrow (BM).

Our laboratory has previously published that G-CSF antagonism ameliorates disease in collagen-induced arthritis.

In this study we investigated the role of G-CSF in a mouse model of uveitis (experimental autoimmune uveitis (EAU)) using G-CSF$^{-/-}$ mice and *in vivo* administration of an anti-G-CSF blocking monoclonal antibody. We demonstrated that intra ocular levels of G-CSF rise dramatically and remain elevated throughout the course of EAU. Disease severity was significantly decreased in both G-CSF$^{-/-}$ mice and anti-G-CSF antibody treated mice. Neutralisation of G-CSF reduced uveitis-induced peripheral blood neutrophilia without inducing significant neutropenia.

Flow cytometric analysis of the wildtype eye showed infiltration by macrophages, T cells and neutrophils. In contrast, analysis of G-CSF$^{-/-}$ eyes revealed no neutrophil infiltrate. To investigate this finding we analysed chemokine receptor expression on peripheral blood neutrophils, which revealed marked alterations in the expression of CXCR2, a receptor known to be required for neutrophil migration into the

eye during uveitis. Furthermore, CXCR2 ligands were elevated in both mouse (KC) and human (IL-8) aqueous and/or vitreous humour uveitis samples. This data suggest that endogenous G-CSF, via its effects on neutrophils, may be a new therapeutic target in uveitis.

ORIA/New Investigator Grant

A national registry of thyroid eye disease for genomic and transcriptomic studies

New Investigator Grant – Jwu Jin Khong

Thyroid orbitopathy (or thyroid eye disease) is a complex disorder that develops in 25% of patients with Graves' disease. The risk factors for developing thyroid eye disease are unclear. We hypothesized that genetic risk factors exist and aimed to elucidate the genetic susceptibility through genetic and transcriptomic approaches. We would like to thank ORIA for supporting this research initiative, which assisted the Flinders University Department of Ophthalmology to further secure an NHMRC project grant (App ID 11031362) commencing in 2012 for genomic association studies to identify major genetic determinants of five blinding eye diseases using pooled DNA, including thyroid eye disease. This NHMRC funding will allow the genetic analysis of samples recruited by this project.

This research project has made excellent progress as follows:

Aim 1: To create a repository of Graves' disease patients with and without thyroid orbitopathy for future genetic studies.

We have recruited a large cohort of well characterized patients with thyroid eye disease and Graves disease in Adelaide and Melbourne totaling 600 cases and controls to date.

The recruitment strategy involved on site recruitment from endocrine and ophthalmology clinics at the Royal Adelaide Hospital and Flinders Medical centre in South Australia, and at the Western Hospital, Sunshine Hospital, Royal Melbourne Hospital and Royal Victorian Eye and Ear Hospital in Melbourne. Mail out invitations to patients in private sectors had also generated reasonable response, with a positive response rate or around 40%. Ethics approval for this project is currently ongoing with provision of annual reports.

Every participant that attended their research appointments had blood taken for DNA stored and 90% also had serum extracted for future research. We have also comprehensively documented the clinical history including known risk factors for developing thyroid eye disease, such as smoking status, cigarette pack years, radioactive iodine treatment exposure, gender, age and ethnicity. These variables will be factored into the future genetic data analysis. We have also carefully document ophthalmic findings using the VISA classification. We have thus assembled one of the best cohorts of thyroid eye disease cases and Graves' controls available for genetic studies.

We have extracted DNA and created DNA pools from the first hundred thyroid eye disease cases and first sixty Graves disease controls in January 2012 for a genome wide association study funded by NHMRC.

Aim 2: Recruitment of patients for tissue specimens for RNA microarray study

We have recruited eighty orbital fat samples from 55 thyroid eye disease cases undergoing orbital decompression surgery and 25 normal controls undergoing non-Grave's related ocular surgery. This collection has exceeded the projected total for orbital fat samples for the study and is now the largest collection of human orbital fat for thyroid eye disease to our knowledge. Previous studies of orbital fat microarrays in the literature to date have evaluated less than 10 samples each. We have maximized the power of the study by reducing RNA variability through matching orbital fat samples for anatomical location, age, gender and ethnicity. As our recruitment strategy has proven, so successful we will have a suitably large sample size for replication studies in the future. The majority of case samples were collected during inactive thyroid eye disease, however six cases underwent surgery during an active disease phase. These cases will be analyzed in an active vs inactive comparison.

In addition to the orbital fat, we have harvested Muller's muscle samples from one case and one control. We plan to use these samples for RNA expression comparison in the future. This would be the first expression study of Muller's muscle which would be entirely novel. We plan to build up the Muller's muscle series over time, but this tissue is less accessible than the orbital fat which has been the focus of this project.

Aim 3: Analyzing the differential RNA expression profile of orbital fat from thyroid eye disease cases and controls

We have extracted total RNA from thirty four orbital fat tissue samples (17 cases and 17 controls, matched for age, sex and ethnicity), using RNeasy microarray kit with the Trizol and chloroform protocol (Qiagen). Quality control of RNA extract was analyzed using RNA 6000 Nano kit and Agilent 2100 Bioanalyzer. The RIN score is a reflection of the quality of total RNA extracted, our samples have on average a RIN score of 6.9 out of 10 and were of sufficient quality to conduct the planned microarray experiments.

We have generated an RNA expression profile for each fat sample using Illumina high throughput RNA microarrays. Analysis has been conducted comparing gene expression in thyroid eye disease cases to normal controls, and also in active thyroid eye disease to inactive thyroid eye disease. Forty-seven genes were differentially expressed in thyroid eye disease compared to controls by more than 1.5 fold at $p<0.05$. We are currently performing pathways analysis on this data to understand the functions and relationships of these differentially expressed genes in order to prioritize genes for further follow-up. The active vs inactive comparison demonstrated a significant enrichment for genes involved in T-cell regulation amongst the 247 differentially expressed genes. This tantalizing result indicates the success to date of this project. These findings will be validated by quantitative real time PCR as planned. We will present the findings of this research at the RANZCO annual scientific meeting in November 2012.

ORIA/RANZCO Eye Foundation Grant

Measurement and monitoring of demyelination *in vivo* using an animal model of optic neuritis

Prof Stuart Graham, Dr Alexander Klistorner and Dr Yuyi You

The aim of this study was to set up a predictable animal model with focal demyelination in the optic nerve, for the study of optic neuritis, and to investigate the relationship between size of the demyelinated optic nerve lesion, extent of axonal loss and degree of visual evoked potential (VEP) delay and amplitude loss.

We have successfully established the rat optic neuritis model using lysolecithin microinjection (You *et al*. IOVS 2011, Figure 1). We have also modified the VEP recording protocol in rats using different designs of implanted skull electrodes, visual stimulators and peak analysis (You *et al*. Doc Ophthalmol 2011).

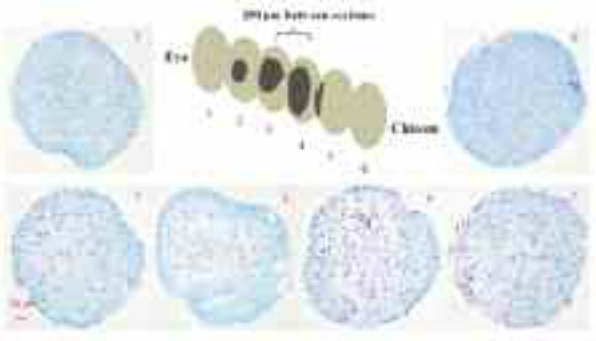
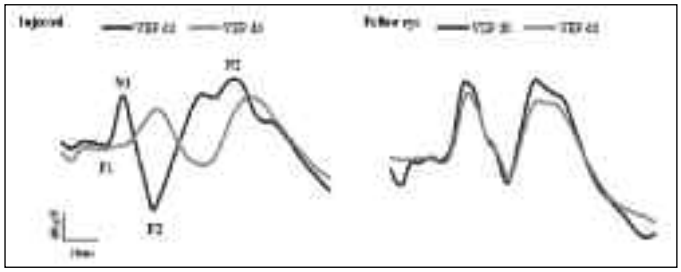


Figure 1: A representative lesion of demyelination in the optic nerve on 250-um serial cross-sections (Luxol-fast blue staining) after lysolecithin microinjection.

A strong linear relationship has been observed between the volume of the demyelinated lesion and the latency delay of VEP (Figure 2 and Figure 3).

Figure 2: Latency delay of visual evoked potential after lysolecithin injection.



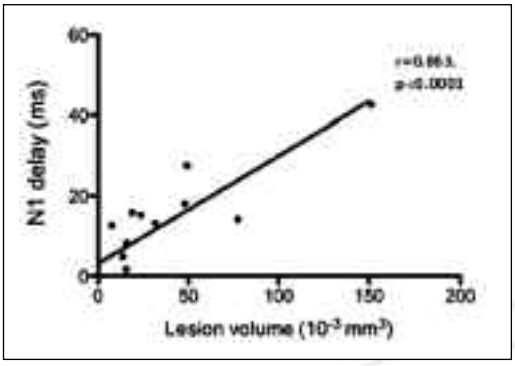


Figure 3: Shows linear relationship between latency delay and demyelination.

In addition, we have improved the reproducibility of VEP amplitude measurement in rats by using a novel electroencephalogram (EEG)-based signal scaling technique (You *et al*. IOVS 2012, Figure 4). After correcting original VEP signals based on underlying EEG levels, we observed a strong correlation between the VEP amplitude decrease and optic nerve axonal loss (You *et al*. IOVS 2012, Figure 5).

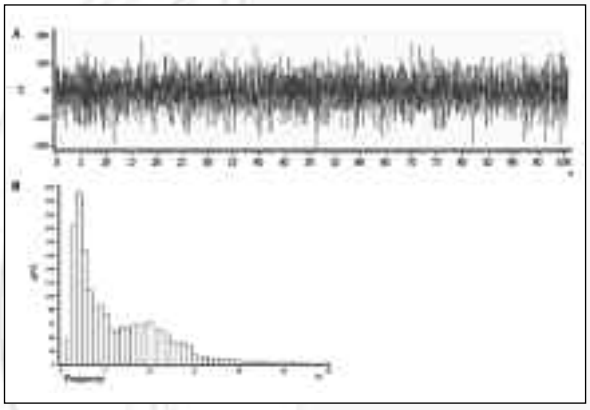


Figure 4: Quantifying electroencephalogram levels based on fast Fourier transform.

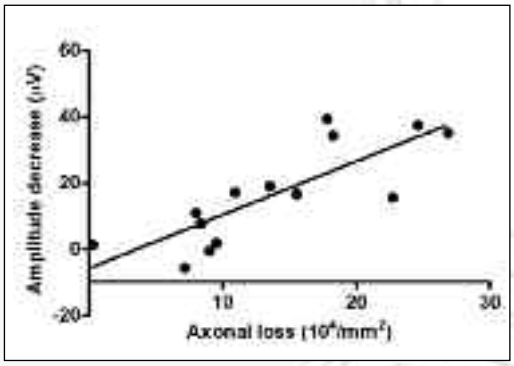


Figure 5: A strong linear relationship between amplitude decrease and axonal loss (EEG-scaled).

The studies confirm *in vivo* that VEP delay is a true measure of the degree of demyelination, while amplitude reduction reflects axonal damage. This supports the concept that the VEP is a useful tool to monitor demyelination and axonal damage in optic neuropathies.

Publications arising from this ORIA project

You Y, Klistorner A, Thie J, Gupta VK, Graham SL. (2012) Axonal loss in a rat model of optic neuritis is closely correlated with visual evoked potential amplitudes using electroencephalogram based scaling. *Invest Ophthalmol Vis Sci*; 53: In Press.

You Y, Thie J, Klistorner A, Gupta VK, Graham SL. (2012) Normalization of visual evoked potentials using underlying electroencephalogram levels improves amplitude reproducibility in rats. *Invest Ophthalmol Vis Sci*; 53:1473-1478.

Gupta VK, You Y, Klistorner A, Graham SL. (2011) Focus on molecules: Sphingosine 1 Phosphate (S1P). *Exp Eye Res*; 10.1016/j.exer.2011.09.023.

You Y, Klistorner A, Thie J, Graham SL. (2011) Improving reproducibility of VEP recording in rats: electrodes, stimulus source and peak analysis. *Doc Ophthalmol*; 123:109-119.

You Y, Klistorner A, Thie J, Graham SL. (2011) Latency delay of visual evoked potential is a real measurement of demyelination in a rat model of optic neuritis. *Invest Ophthalmol Vis Sci*; 52: 6911-6918.

You Y, Klistorner A, Graham SL. (2012) Visual Evoked Potential Recording in Rodents. In *Neuromethods*. Stimulation and inhibition of neurons. Pilowsky PM (ed). Berlin, Humana Press.

ORIA/Renensson Bequest Grant

Do primary cilia provide the key to promoting regeneration of a transparent lens after cataract surgery?

Prof John McAvoy & A/Prof Frank Lovicu

Overall aim

The primary cilium is an antenna-like structure that protrudes from the outer surfaces of most cells. Recent studies shave shown that it acts as part of Global Positioning System (GPS) that guides cells to their correct destination. Recently we showed that lens cells have primary cilia and now we want to determine if they can respond to guidance cues to facilitate lens regeneration after cataract surgery.

The research plan was in two parts. In *Part 1*, the aim was to study the development and distribution of primary cilia during FGF-induced fibre differentiation in explant cultures. Given confirmation that cilia consistently localise to the tips of the elongating fibres that abut the epithelium (fits our model of an epithelial/primary cilia interaction in aligning/orienting fibres), in *Part 2* of the project, we will then assess experimentally the functional importance of this relationship. For this we will determine if such fibre alignment/orientation still occurs in explants from lenses of mice that are deficient in functional primary cilia.

Progress

Part 1 – Our recent studies have shown that when lens epithelial cells shift from the germinative zone and begin to elongate in the transitional zone, they exhibit Planar Cell Polarity (PCP) as shown by translocation of their primary cilium/centrosome to the leading edge of each elongating fiber cell, i.e. the side of the cell at its apex that faces the anterior pole. This is also accompanied by similar polarization of Frizzled 6 (Fz6) and some other PCP proteins, Fz3, Vang-Gogh-like and inversin. To determine if a similar translocation of primary cilium/centrosome and Fz was a feature of FGF-induced fiber differentiation, we localized pericentrin and Fz6 in our explants. Note that pericentrin is a major component of the centrosome that in many cell types, and certainly in lens cells, is associated with the primary cilium at the apical margin of the cell. In controls, Fz6 localizes to small, variable-sized domains at the cell margins. In elongating fibers, strong Fz6 reactivity becomes prominent at one end of the cell. We identified this as the apical tip (or leading

edge) of the elongating fibers because this is where the centrosomal marker, pericentrin, is also localized. In controls, a spot of pericentrin immuno-reactivity is located next to the nucleus and exhibits no spatial relationship with the Fz6 domain; however, during fiber differentiation pericentrin becomes localized next to Fz6 at the apical cell margin. This shows that a close spatial relationship develops between Fz6 and the centrosome/primary cilium during FGF-induced fiber differentiation in explants and this mimics the relationship that is evident at the apical tip of each fiber cell *in vivo*.

During FGF-induced fibre differentiation, groups of cells often show similar alignment. Pericentrin immunoreactivity also shows that in many cases these groups of cells exhibit similar orientation. Further analysis of such groups of cells, when we were able to get optical sections obliquely or transversely through them, shows that many have hexagonal profiles and are regularly packed, similar to fibers *in vivo*. In addition, in most of the cells within these groups, pericentrin reactivity is clearly located to the same side of each cell and is often surrounded by stabilized microtubules. This shows that, during FGF-induced fiber differentiation, groups of cells assume a similar alignment/orientation that is characteristic of lens fibers *in vivo*. As noted previously, in most cases these similarly oriented/aligned cells were polarized towards groups of smaller cells, i.e. cells that had failed to elongate and were present in compact clusters. We often found that when these groups, or islands of cells were well developed, the elongated cells were highly ordered. Whilst the cellular arrangements varied considerably, one thing that was consistent was the alignment of the elongating fibers at approximately right angles to a group, or island, of smaller cells. The elongated cells were readily visualized by immunlocalisation of acetylated tubulin. We also confirmed that the fibers were similarly polarized as the bright spot of pericentrin reactivity that demarcates the apical tip of each cell was similarly localized in these groups of elongating cells and mostly this was juxtaposed to the clusters of smaller cells.

These studies clearly showed polarization of the centrosome; however, the primary cilium as localized by γ-tubulin immuno-reactivity was substantially reduced and sometimes not detectable in these differentiating fibres. This result appears to go against our initial hypothesis that the cilium detects a GPS from the epithelium. Instead, it is Fz that is upregulated and becomes translocated to the leading edge of the elongating fibres. Thus, our current view is that it is Fz that is responding to the epithelial-derived polarizing cue (perhaps a member of the Wnt family of Fz ligands) and that the primary cilium has little or no function. Further indications that the cilium may have no functional role in fibre differentiation comes from our cilia knockout studies (funded by other grants) that show normal fibres with normal alignment and orientation in mice that have cilia conditionally knocked out in lens from about embryonic day 13.5.

Because this result shows that loss of the primary cilium has no adverse effects on differentiation of fibres, the ***Part 2*** of the project that we had planned (to assess the functional importance of the primary cilia in aligning/orienting differentiating fibres towards epithelial cells) became redundant. Instead, because of the apparent involvement of Wnt-Fz and the Wnt-Fz/PCP signalling pathway in FGF-induced fibre differention, we focused our efforts on investigating its role in fibre differentiation. Given that FGF triggers all the morphological and molecular events in fibre differentiation we set out to determine if Wnt-Fz/PCP signalling is activated by FGF and if it plays a central role in regulating the cytoskeletal dynamics that underlie this process. In support of this we have shown that FGF upregulates Wnt-Fz/PCP signalling components and enhances the activity of this pathway. Consistent with this, the introduction of Wnt-Fz signalling inhibitors blocks FGF-induced fibre cell elongation and ß-crystallin accumulation, both key differentiation markers. Coinciding with this we also show that Wnt-Fz signalling activity is reduced. Taken together this provides compelling evidence that this pathway plays a key role downstream of FGF in the fibre differentiation process.

In conclusion, ORIA support has helped us understand better the molecular mechanisms behind our recent discovery that the FGF-induced fibre differentiation response involves promotion of Wnt-Fz/PCP signalling and this may have a role in regulating polarized cell behaviour that, in the presence of epithelial cells, results in their aligned orientation similar to that seen in vivo. This provides key insights into the factors and conditions that are needed to promote self-assembly of lens cells into structures resembling those *in vivo* and serves to emphasize the importance of understanding the interactions between the different cell types that normally occur within a tissue/organ. This is fundamental to defining the specific conditions and stimuli needed to promote the inherent capacity of lens cells to recapitulate developmental programs in vitro and assemble into a functional lens.

Publications related to this work

Sugiyama Y, Lovicu FJ, McAvoy JW. Planar cell polarity in the mammalian eye lens. *Organogenesis* 2011;7:191-201.

Sugiyama Y, McAvoy JW. Analysis of PCP defects in mammalian eye lens. In: *Planar Cell Polarity Signalling*. *Methods Mol Biol*. 2012;839:147-56.

ORIA/Ida Mann Grant

To understand the cause of a blinding corneal disease: Fuchs' endothelial dystrophy

Dr S Sharma, Dr T Chataway, Dr B Llamas, Dr GR Snibson, Dr R Mills

Overview

Fuchs' corneal endothelial dystrophy (FCED) is a progressive degenerative disease of the cornea, generally prevalent in the elderly population. It is one of the most common indications for corneal transplantation. Delay in timely treatment due to waiting times for transplantation surgery in these patients can negatively impact quality of life. The objective of this project is to understand the molecular mechanisms underlying FCED to allow development of strategies for early diagnosis, prevention and/or management of the disease, in the future.

Background

FCED is an ocular condition that affects the posterior cornea, particularly the Descemet's membrane and the corneal endothelium. The characteristic abnormalities include thickening of the Descemet's membrane accompanied by posterior outgrowths, clinically referred to as 'guttae', and thinning and gradual loss of corneal endothelial cells. In advanced cases these changes lead to corneal edema and opacification that in turn can lead to severe vision impairment and cause pain. The anterior cornea is also affected in advanced cases involving epithelial erosion. FCED is a progressive disease and can have an early or late onset. The early-onset form is rare while the late-onset form more common. The advanced cases require corneal transplantation. In the recent years, partial thickness grafts namely, deep lamellar endothelial keratoplasty (DLEK) or Descemet stripping endothelial keratoplasty (DSEK), have been more commonly performed in these patients. FCED is one of the most common causes of corneal transplantation in Australia. It is a complex disease with strong hereditary component. The early-onset form is a familial disease, while the late-onset form can be familial or non-familial (sporadic). The sporadic late-onset form is the most common. Mutations in the *COL8A2* gene have been reported to cause early-onset disease while mutations in *SCL4A1*1 and *TCF8* genes cause late-onset disease. Recently, ours and other groups have reported polymorphisms in the *TCF4* gene to increase the risk of late-onset FCED. A few molecular investigations have been undertaken in this disease, these indicate the involvement of epithelial-to-mesenchymal transition, oxidative stress and cellular apoptosis.

The aims of this project are, 1) to identify protein constituents of the abnormal Descemet's membrane in FCED, and 2) to identify genes differentially expressed in FCED-affected compared to unaffected corneal endothelium.

Progress to date

Identification of protein constituents of Descemet's membrane in FCED: Proteins from DSEK specimens from FCED patients were extracted using a combination of chemical cleavage and chaotropic agents and tryptic peptides analysed by mass spectrometry. Peptides corresponding to a total of 20 different proteins were detected with significant probability scores. Generally, multiple peptides corresponding to a protein were detected. Several of the proteins were identified in three or more of the five analysed samples. Clusterin, TGFBI (Transforming growth factor-induced), HSPG2 (Heparan sulfate proteoglycan-2),

keratocan, thromspondin-1, HTRA1 (High-temperature requirement A serine peptidase 1), collagen 8A1, 8A2 and 12A1, laminin subunits, emilin-1 and apolipoprotein E were among the most readily identified proteins. Clusterin and TGFBI have been reported to be upregulated in FCED-affected corneas and mutations in *COL8A1* have been reported in early-onset FCED. Clusterin is a molecular chaperone, implicated in protecting cells from physiological stresses caused by aging, oxidative stress and apoptosis. TGFBI is a secreted extracellular matrix protein that mediates cell adhesion by interacting with other extracellular matrix components such as collagens. Mass spectrometry results were validated by immunohistochemical labelling using sections of affected and unaffected corneas. The data showed difference in distribution of clusterin and TGFBI proteins in the affected compared to unaffected corneas. Clusterin expression was seen in the corneal epithelium and anterior phase of the thickened Descemet's membrane in affected corneas. This pattern was not evident in normal corneas. These results are consistent with corneal epithelial oedema and possibly impaired secretion of clusterin expressed by corneal endothelial cells into the posterior Descemet's membrane in FCED. Expression of TGFBI was seen in the corneal epithelium and the thickened Descemet's membrane but was conspicuously absent in the Bowman's membrane in affected cornea. In the normal cornea the protein was not expressed in the corneal epithelium but was present both in the Bowman's and Descemet's membrane. The absence of TGFBI in the Bowman's membrane is consistent with the reported depletion of stromal keratocytes in the affected corneas. Expression of clusterin and TGFBI in the corneal epithelium possibly indicates a stress response. These findings have been published in the peer-reviewed literature. Validation of the other identified proteins of interest is underway.

Identification of genes differentially expressed in the corneal endothelium in FCED: Corneal endothelial RNA was extracted from DSEK specimens from FCED patients and equivalent specimens from normal deceased donors. Microarray analysis was performed on RNA from three pooled (n=3 per pool) affected and three individual unaffected samples using the Illumina Human HT-12 expression array. 110 genes were differentially expressed (Bonferroni corrected p value <0.05) at ≥ 10 fold change. Ingenuity Pathway Analysis (IPA) of these genes indicates significantly high probability of their representation in 6 inter-related functional networks in the Ingenuity Knowledge Base. The networks and corresponding confidence scores are shown in Table 1. Differential expression of genes involved in cellular growth, proliferation, morphology and movement correlates with involvement of epithelial-to-mesenchymal transition in FCED. The analysis also indicates differential expression of genes involved in inflammation, oxidative stress and apoptosis in corneal endothelium in FCED. These results will guide prioritisation of genes for validation of differential expression. Validation will be performed by real-time RT-PCR on independent affected and unaffected samples. The results of this Aim were presented at the local ASMR conference.

Top functions	Score
Cardiovascular Disease, Cellular Growth and Proliferation, Cellular Development	43
Cell Morphology, Inflammatory Disease, Neurological Disease	42
Free Radical Scavenging, Carbohydrate Metabolism, Cell Death	27
Cellular Movement, Haematological System Development and Function, Immune Cell Trafficking	26
Cancer, Connective Tissue Disorders, Developmental Disorder	24
Antigen Presentation, Haematological System Development and Function, Inflammatory Response	21

Table 1. The top network functions representing differentially genes expressed in FCED-affected corneal endothelium in the Ingenuity Knowledge Base.

Publication arising from this work

A Kuot, AW Hewitt, K Griggs, S Klebe, R Mills, V Jhanji, JE Craig, S Sharma, KP Burdon. (2012) Association of *TCF4* and *CLU* polymorphisms with Fuchs' endothelial dystrophy and implication of CLU and TGFBI proteins in the disease process. *Eur J Hum Genet*. 20(6):632-8.

Conference presentation arising from this work

A Kuot, R Mills, G Snibson, KP Burdon, JE Craig and S Sharma. Differential gene expression analysis of human corneal endothelium in Fuchs' endothelial corneal dystrophy. ASMR SA Annual Scientific Meeting, June 6, 2012.

ORIA/RANZCO Eye Foundation Grant

Do transplants of corneal endothelium undergo rejection?

Prof DJ Coster, Dr S Klebe and Prof KA Williams

Overview, hypothesis and aim

The question we have been addressing is whether corneal endothelial cell transplants are more or less susceptible to graft rejection than are conventional corneal transplants. We developed a new animal model akin to DSEK, to investigate the impact of rejection on the engraftment of endothelial transplants. Our *hypothesis* was that endothelial transplants are subject to allograft rejection. Our *aim* was to test this hypothesis in a sheep model of penetrating transplantation and endokeratoplasty.

Background

Component endothelial cell transplantation, or endokeratoplasty, is widely used for bullous keratopathy and for Fuchs' endothelial dystrophy. The rationale for endokeratoplasty is that in conditions in which only the corneal endothelium is dysfunctional or absent, then only this component of the cornea needs to be replaced. The benefits claimed for endokeratoplasty in its various forms are reduced post-operative suture complications, inflammation and astigmatism, increased wound strength, and faster visual rehabilitation compared with penetrating keratoplasty. An important unresolved issue is the extent to which endothelial allografts undergo rejection, and therefore the extent to which post-operative topical immunosuppression needs to be administered. Many of the essential elements that initiate an allograft response – blood vessels, lymphatics, and antigen-presenting cells – are located within the corneal stroma. In penetrating keratoplasty, all of these elements are of donor origin, so that the foreign antigenic load is relatively high. Indeed, the major cause of the failure of penetrating corneal grafts is irreversible rejection. The anterior chamber, in contrast, is an immune-privileged site in which allogeneic skin grafts are not necessarily rejected. Endothelial grafts are placed into the anterior chamber, and the pattern of rejection might conceivably differ from that seen with penetrating keratoplasty.

We considered the outbred sheep to be a suitable outbred preclinical model in which to test the relative rejection rates of penetrating and component endothelial cell corneal grafts. The ovine endothelium is non-replicative, as in humans, and the anatomy of the eye is similar to that of the human, with a deep anterior chamber. In the absence of topical immunosuppression, penetrating ovine corneal allografts undergo rejection several weeks after transplantation in a manner that is similar clinically and histologically to corneal allograft rejection in humans. We developed a method of endokeratoplasty in the sheep that is similar to human Descemet's stripping endothelial keratoplasty (DSEK). We then performed contemporaneous ovine penetrating and endothelial cell allografts, to examine the clinical and histological outcomes, in particular, the relative rates of rejection, in the absence of any confounding immunosuppression.

Progress to date

Penetrating corneal allografts (n = 7) achieved perfect transparency after surgery more quickly than did endothelial cell allografts, at a median of 4 days (range 1-6 days) for penetrating grafts and 10 days (range 4–31 days for endothelial grafts), $p = 0.0034$. Six of seven penetrating grafts underwent rejection at a median of 18 days (range 16–29 days) after surgery (Table 1). Graft rejection was accompanied by inflammation and neovascularization of the graft, and grafts swiftly opacified. The one graft that survived for more than

60 days post-graft developed a hyphaema at day 16 and was somewhat cloudy from day 25 to day 59, indicative of a rejection episode, although it never completely clouded. *All* ovine endokeratoplasties underwent rejection, as assessed by complete corneal opacification but at a significantly slower pace than did the penetrating corneal grafts, with a median time to rejection of 48 days (range 19->60), p = 0.036.

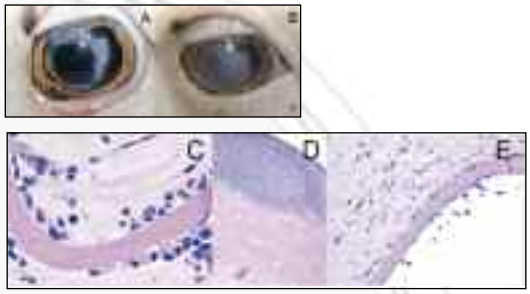
The rejection process in the penetrating and lamellar graft procedures was different. Eyes with an endothelial cell graft *in situ* did not become significantly inflamed. The process leading to loss of corneal clarity was often indolent, with fluctuating oedema. Keratic precipitates and small rejection lines became visible at the slit lamp and subsequently disappeared, only to reappear several days later. However, once the iris margins were no longer clearly visible through the graft, then complete failure over the following few days was inevitable (Fig. 1A, B).

Histopathologic correlates were made on H&E sections. Of the seven penetrating grafts, six showed clear evidence of rejection, as evidenced by central stromal neovascularization, infiltration of the limbus, stroma and sometimes epithelium with mononuclear cells, mononuclear cells in the anterior chamber, stromal oedema, and missing endothelium (Fig. 1C). The exception showed some evidence of corneal neovascularization and a sparse leukocytic infiltrate, but the corneal endothelial monolayer appeared intact. Several of the rejected grafts exhibited semi-organised accumulations of lymphoid cells that resembled germinal centres (Fig. 1D). Of the ten endokeratoplasties, three showed evidence of an indolent rejection process, marked by deep neovascularization of the stroma, and a slight to moderate infiltrate of mononuclear cells. The endothelial monolayer was mostly intact. In a further 5 grafts, the evidence of rejection was pronounced (Fig. 1E).

Fig. 1. Outcomes of ovine keratoplasty:

A) transparent endokeratoplasty at day 41 post-graft;
B) same eye at day 48 post-graft: the graft is failing. H&E sections of rejected grafts;

C) endothelial rejection in an eye with a penetrating graft;
D) semi-organised lymphoid follicle at limbus in eye with a rejected penetrating corneal graft;
E) endothelial rejection in an endokeratoplasty.



Summary

One of the potential benefits of the alternative surgical procedure of endokeratoplasty is that rejection may be less of a concern than for penetrating keratoplasty. However, we found that in the absence of any topical or other immunosuppression, all of the ovine endothelial cell grafts in our series underwent rejection, albeit at a slower tempo than did contemporaneous penetrating grafts. What are the implications for human endothelial cell grafts? Sensitisation to foreign alloantigen is very likely to occur eventually, especially if the recipient is at moderate to high risk of rejection by virtue of a co-morbidity. Topical immunosuppression may need to be continued in the longer term. A manuscript is currently being prepared for submission.

Publications arising from ORIA support for projects in the recent past

Brice SL, Kirk K, Brereton HM, Coster DJ, Williams KA. The influence of cervical and thoracic lymphadenectomy on corneal allograft rejection in inbred rats. *Br J Ophthalmol* 2011, *Br J Ophthalmol* 2012; 96: 448-50.

Williams KA, Klebe S. Gene therapy for corneal dystrophies and disease, where are we? *Curr Opin Ophthalmol* 2012 (in press).

Some of our researchers

Dr Kathryn Burdon

Dr Ian Wicks and Dr Gabrielle Goldberg

Dr Vicki Chrysostomou

Dr Karl Brown

A/Prof Jamie Craig

Directors' Report

For the year ended 30 June 2012

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 30 June 2012 and report as follows:

1. Directors

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

Professor Stuart Graham, Sydney (Chairman)
Professor Mark Gillies, Sydney (Vice Chairman)
Dr Richard Mills, Adelaide (Honorary Secretary)
Dr Wilson Heriot, Melbourne (Honorary Treasurer)
Dr Fred Chen, Perth
Dr Colin Clement, Sydney
Professor Jonathon Crowston, Melbourne
A/Prof Mark Daniell, Melbourne
Dr Paul Healey, Sydney
Dr Anthony Kwan, Brisbane
Professor David Mackey, Perth
Profesor Peter McCluskey, Sydney
Dr John Males, Sydney
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 (resigned Nov 2011)		2005
Dr Fred Chen, MB BS(Hons), PhD(Lond), FRANZCO, CSA(Cert)		2011
Dr Colin Clement, BSc(Hons), MBBS, PhD, FRANZCO		2011
Professor J Crowston, BSc, MBBS, FRCOphth, FRANZCO, PhD		2008
A/Prof Mark Daniell, MB BS, MS, FRACS, FRANZCO		2001
Professor Mark Gillies, MB BS, PhD, FRANZCO	Vice Chair	2004
Professor Stuart Graham, MB BS, MS, FRANZCO, FRACS	Chair	2001
Dr Paul Healey, MBBS(Hons), B(Med)Sc, MMed, PhD, FRANZCO		2011
Dr Wilson Heriot, MBBS, FRANZCO, FRACS	Hon Treasurer	2009
Dr Anthony Kwan, MBChB(UK), MD(Lond), 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, M Med, FRANZCO		2009
Dr Richard Mills, MB BS, FRCS, FRACS, FRANZCO, PhD	Hon Secretary	2003
Dr Andrea Vincent, MBChB, FRANZCO		2008
Dr Stephanie Watson, BSc, MBBS, FRANZCO, PhD		2006
Professor Tien Wong, MB BS, MPG, PhD, FRANZCO		2008

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	2	2
Dr Fred Chen	1	0
Dr Colin Clement	1	1
Prof Jonathon Crowston, Melbourne	3	2
A/Prof M Daniell, Melbourne	3	2
Prof Mark Gillies, Sydney	3	2
Prof Stuart Graham, Sydney	3	3
A/Prof Paul Healey, Sydney	1	1
Dr Wilson Heriot, Melbourne	3	3
Dr Anthony Kwan, Brisbane	3	2
Prof David Mackey, Perth	3	3
Prof P J McCluskey, Sydney	3	2
Dr J Males, Sydney	3	2
Dr Richard Mills, Adelaide	3	3
Dr A Vincent, New Zealand	3	3
Prof Tien Wong, Melbourne	3	0
Dr Stephanie Watson, Sydney	3	3

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. Short-term and Long-term Objectives

The company's short-term objectives are to:

- continue to fund research into all types of eye diseases annually in Australia
- continue to be in the forefront of advancing eye research in Australia
- continue to suport the presentation of research and the publication of the results of researdh for vision scientists and ophthalmologists for the benefit of all Australians
- continue to support new scientists by providing a percentage of its annaul funding to support this category.

The company's long-term objectives are to:

- increase the funds available for the provision of research funding in order to achieve its mission statement of advancing eye researh in Australia
- ensure that the funding it provides leads to researchers gaining a track record to enable them to secure larger grants towards bigger and successful projects.

7. Strategies

To achieve its stated objectives, the company has adopted the following strategies:

- The company is partnered with the RANZCO Eye Foundation which is now primarily responsible for raising additonal funding towards the ORIA's research projects and to raise awareness generally of eye health within Australia.
- The company's Investment Advisory Committee monitors and works towards successfully managing the company's invested funds, the profits from which are used annually for research funding.
- The company connects with other vision related organisations in Australia to support funding of projects for specific diseases.
- The company strives to attract, support and retain quality staff who are committed to the work of the organisation.
- The company conducts audits on its previously funded resarch to ensure the funding it provides is meeting its objectives.
- The company's Board and Research Advisory Committee is made up of leading vision scientists and ophthalmologists within Australia.

8. Operating Results

(1) Operating Revenue

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

	2012	2011	Increase	%
Net dividend interest and trust distribution income	$620,024	$585.578	$34,446	5.88
Less Expenses	33,549	35,777		
	$586,475	$549,801		

(2) Operating Surplus

The net surplus of the company before other comprehensive income for the year ended 30 June 2012 was $613,881 (2011: $561,217. This amount is comprised of the following:

	2012	2011
Trust Fund	$613,881	$561,217
Administration	8,995	(5,031)
	$662,876	$556,186

Other comprehensive loss before grants and Director of Research allocation amounted to a loss of $566,289 (2011: surplus of $325,974 and included a loss on re-arrangement of investments of $265,020 (2011: loss of $143,671, special dividends and associated imputation credits of $nil (2011: $448,611) and valuation losses on available-for-sale financial assets of $301,269 (2011: gain of $21,034).

9. Review of Operations

The surplus for the year was $622,876 compared to $556,186 in 2011. The income of the trust fund increased by $20,436 mainly due to an increase in dividends from investments in company shares. Distributions from legacies and donations increased to $26,624 from $40,854 in 2011. The administrative operations of the Institute for the year resulted in a surplus of $8,995 compared with a deficit of $5,031 in 2011.

10. Dividends

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

11. State of Affairs

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

12. 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.

13. Share Options

No share options were issued during the year.

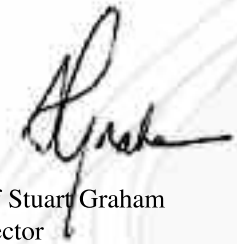
14. Directors' Benefits

With the exception of the grants made or allocated to Assoc. Professor Robert Casson, Professor Stuart Graham, Assoc. Professor Mark Daniell and Dr Richard Mills, 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.

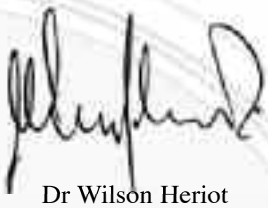
15. Auditor's Independence Declaration

A copy of the auditor's independence declaration as required under Section 307 C of the Corporations Act 2001 is set out at page 47.

For and on behalf of the Board.



Prof Stuart Graham
Director



Dr Wilson Heriot
Director

Sydney
Signed in accordance with a resolution of directors,
this 22nd day of September 2012

Statement of Financial Position

As at 30 June 2012

	Note	2012 $	2011 $
Current Assets			
Cash and Cash Equivalents	3	957,903	860,199
Receivables	4	144,665	353,250
Investments	5	7,626,966	8,059,214
		8,729,534	9,272,663
Non-Current Assets			
Plant & Equipment	6	5,546	150
Total Assets		8,735,080	9,272,813
Current Liabilities			
Payables	7	481,817	551,355
Provision	8	19,515	15,607
		501,332	566,962
Net Assets		8,233,748	8,705,851
Equity			
General Fund	13 (a)	–	–
Capital Funds			
Research Fund	9	941,118	928,557
Settled Funds	10	472,556	472,556
Financial Assets Reserve	11	43,699	344,968
Capitalised Profit on Re-arrangement of Investments and Capital Distributions	12	6,323,599	6,588,619
		7,780,972	8,334,700
Retained Income – available for grants	13 (b)	452,776	371,151
Total Equity		8,233,748	8,705,851

The accompanying Notes form part of these financial statements.

Trust Fund Statement of Comprehensive Income

For the year ended 30 June 2012

	Note	2012 $	2011 $
INCOME			
Dividends received from:			
Other corporations		504,348	456,987
Total Dividends		504,348	456,987
Interest received from:			
Other entities		88,303	89,156
Trust distributions received from:			
Other entities		27,373	39,433
		620,024	585,576
Legacies – Anselmi Estate		19,556	34,751
– Ivy May Stephenson		5,495	5,603
Other donations and legacies received		1,573	500
Sundry Income		782	564
Total Income for year		647,430	626,994
EXPENSES			
AOVS meeting contribution		–	10,000
Donation – Singapore Eye Research Institute		–	20,000
Commission paid		33,549	35,777
		33,549	65,777
SURPLUS FOR THE YEAR		613,881	561,217
Other Comprehensive Income			
Special dividends and associated imputation credits		–	448,611
Valuation Gains/(Losses) on available-for-sale financial assets		(301,269)	21,034
Profit/(Loss) on re-arrangement of investments		(265,020)	(143,671)
Total other comprehensive income		(566,289)	325,974
Surpus for the year before allocation		47,592	887,191
Grants allocated/made during the year	14	342,690	321,718
Allocation to Director of Research – Victoria	15	186.000	171,000
		528,690	492,718
TOTAL COMPREHENSIVE INCOME/(LOSS)		(481,098)	394,473
Profit attributable to Members of the Entity		85,191	68,499
Total other comprehensive income attributable to Members of the Entity		(566,289)	325,974

The accompanying Notes form part of these financial statements.

Statement of Changes in Equity

for the year ended 30 June 2012

	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 2010	–	917,800	472,556	6,283,678	323,934	318,440	8,316,408
Profit for Year	(5,031)	–	–	–	–	68,499	63,468
Total Other Comprehensive Income	–	–	–	304,941	21,034	–	325,975
Transfers to/(from) Reserves	5,031	10,757	–	–	–	(15,788)	–
Transferred to Profit for the Period	–	–	–	–	–	–	–
Balance at 30 June 2011	–	928,557	472,556	6,588,619	344,968	371,151	8,705,851
Balance at 1 July 2011	–	928,557	472,556	6,588,619	344,968	371,151	8,705,851
Profit for Year	8,995	–	–	–	–	85,191	94,186
Total Other Comprehensive Income	–	–	–	(265,020)	(301,269)	–	(566,289)
Transfers to/(from) Reserves	(8,995)	12,561	–	–	–	(3,566)	–
Transferred to Profit for the Period	–	–	–	–	–	–	–
Balance at 30 June 2012	–	941,118	472,556	6,323,599	43,699	452,776	8,233,748

The accompanying Notes form part of these financial statements.

Administration Statement of Comprehensive Income

for the year ended 30 June 2012

	Note	2012 $	2011 $
INCOME			
Membership Fees – RANZCO		130,162	120,720
Total income		130,162	120,720
EXPENSES			
Accountancy Fees		22,108	23,999
Auditors' Remuneration	16	4,950	4,950
Bank Charges		124	190
Depreciation		689	90
General Expenses		3,576	4,840
IT and Webpage Expenses		392	719
Insurance		4,59	4,149
Printing and Stationery		7,482	11,677
Staff Salaries		53,550	52,937
Superannuation Contribution		6,600	6,230
Salary Sacrificed Benefits		1,450	1,200
Provision Employee Benefits		3,908	2,300
Meeting and Travelling Expenses		12,179	12,470
Total Expenses		121,167	125,751
SURPLUS/(DEFICIT) FOR THE YEAR	13 (a)	8,995	(5,031)
Other Comprehensive Income		–	–
Total Comprehensive Income		8,995	(5,031)

The accompanying Notes form part of these financial statements.

Cash Flow Statement

for the year ended 30 June 2012

	Note	2012 $	2011 $
Cash Flows from Operating Activities			
Receipts			
Dividends Received		712,934	335,654
Interest Received		88,303	89,156
Trust Distributions		27,373	39,433
Legacies		25,051	40,354
Other Revenue		5,454	5,674
RANZCO – Reimbursement of membership fees		130,162	120,720
Contributions from RANZCO Eye Foundation		120,000	100,350
Contribution from Glaucoma Australia Inc		72,005	63,332
Donations received		1,573	500
Payments			
Commissions		(33,5549)	(35,777)
Research Grants Paid		(619,997)	(578,706)
Payments to Director of Research – Victoria		(171,000)	(144,000)
Other Grants and Contributions		–	(30,000)
Other		(120,480)	(121,327)
Net Cash (Used in)/Provided by Operating Activities	17	237,830	(114,637)
Cash Flows from Investing Activities			
Payments for property, plant and equipment		(6,085)	–
Special Dividends – Capital Reduction		–	314,027
Proceeds from Re-arrangement of Investments		5,049,368	6,221,695
Payments for Investments		(5,183,409)	(6,940,814)
Net Cash Used in Investing Activities		(140,126)	(405,092)
Net Increase/(Decrease) in Cash and Cash Equivalents		97,704	(519,729)
Cash and Cash Equivalents at 1 July 2011		860,199	1,379,928
Cash and Cash Equivalents at 30 June 2012	3	957,903	860,199

The accompanying Notes form part of these financial statements.

Notes to and forming part of the Financial Statements
for the year ended 30 June 2012

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 retained income available for grants.

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 retained income available for grants.

(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 company 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.

	2012 $	2011 $
3 Cash and Cash Equivalents		
General Account	809,193	760,403
Donations Account	29,565	42,943
D W Research Fund Account	119,145	56,853
	957,903	860,199
4 Receivables		
Sundry Debtors	144,665	353,250
	144,665	353,250
5 Investments		
Shares in listed corporations and other securities	7,391,966	7,754,214
Total available-for-sale financial assets	7,391,966	7,754,214
Held-to-maturity investments		
Bank Bills – at cost	235,000	305,000
Total held-to-maturity investments	235,000	305,000
Total Investments	7,626,966	8,059,214

	2012 $	2011 $
6 Plant and Equipment		
Office equipment – at cost	8,373	2,288
Less: Accumulated depreciation	(2,827)	(2,138)
	5,546	150
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	150	240
Additions	6,085	–
Less: Depreciation expense	(689)	(90)
Carrying amount at end of year	5,546	150
7 Payables		
Creditors and Accruals	28,469	27,705
Grants Payable	267,348	352,650
Director of Research – Victoria (refer note 15)	186,000	171,000
	481,817	551,355
8 Provisions		
Employee Benefits	19,515	15,607
9 Research Capital Fund		
General		
Balance 1 July 2011	607,202	596,445
Allocation to Capital:		
– 5% Surplus & Imputation Credits & Other Legacies	12,561	10,757
Balance 30 June 2012	619,763	607,202
Anselmi Estate		
Balance 1 July 2011	290,979	290,979
Allocation during year	–	–
Transfer during year	–	–
Balance 30 June 2012	290,979	290,979
Ivy May Stephenson Estate		
Balance 1 July 2011	30,376	30,376
Allocation during the year	–	–
Transfer during year	–	–
Balance 30 June 2012	30,376	30,376
Total	941,118	928,557
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

	2012 $	2011 $
11 Financial Assets Reserve		
Balance 1 July 2011	344,968	323,934
Revaluation increment/(decrement)	(301,269)	21,034
Balance 30 June 2012	43,699	344,968

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

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

	Balance 30/06/2011 $	Allocation of Realised Profit/(Loss) on Re-arrangement of Investments and Capital Distributions $	Balance 30/06/2012 $
Research Fund:			
General	125,801	(6,614)	119,187
Anselmi Estate	45,060	(2,369)	42,691
Ivy May Stephenson	116	(6)	110
D.W. Research Funds	4,882,515	(175,129)	4,707,386
Esme Anderson	886,814	(46,204)	840,610
G.J. Williams	152,263	(8,000)	144,263
B. Mitchell	150,327	(8,000)	142,327
Dame Ida Mann	212,379	(11,237)	201,142
Ronald & Lois Lowe	133,344	(7,461)	125,883
	6,588,619	(265,020)	6,323,599

	Note	2012 $	2011 $
13 Accumulated funds			
(a) Administration			
Accumulated Deficits – 1 July 2011		–	–
Total Comprehensive Income		8,995	(5,031)
Total available for appropriation		8,995	(5,031)
Aggregate of amounts transferred from Administration	13(b)	(8,995)	5,031
Accumulated Deficits – 30 June 2012		–	–
(b) Trust Fund			
Retained Income – 1 July 2011		371,151	318,440
Total Comprehensive Income		85,191	68,499
Total available for appropriation		456,342	386,939
Aggregate of amounts transferred to General/Capital Funds:			
Administration	13(a)	8,995	(5,031)
Research Trust		(12,561)	(10,757)
Retained Income – 30 June 2012		452,776	371,151

	2012 $	2011 $
14 Grants Allocated/Made During the Year		
Dr A Cornish, Dr L Lim and Dr I Wicks		49,900
Prof Paul Baird		40,000
Prof D Coster, Dr S Klebe and Prof K Williams		48,000
Prof R Casson		30,000
Prof S Graham, Dr A Klistorner and Dr Y You		45,000
Dr K Brown, Prof M Daniell, Dr K Abberton and Dr B Ozcelik		49,500
Prof J McAvoy & A/Prof F Lovicu		45,000
Dr V Chrysostomou		46,000
Dr K Burdon, A/Prof J Craig, Dr J Muecke, Dr A Rudkin and Dr J Nicholl		48,000
Dr S Sharma, Dr T Chataway, Dr B Liamas, Dr G Snibson and Dr R Mills		40,000
Dr J Khong		44,000
Dr Alex Hewitt & Dr Stuart Macgregor	46,270	
Dr Glyn Chidlow	49,960	
A/Prof Jamie Craig and Dr David Dimasi	49,250	
Dr John Wood	49,725	
Dr Shiwani Sharma, Dr Kathryn Burdon and Prof Jozef Gécz	50,000	
Dr Rohan Essex, Dr Willie Campbell, Dr Alex Hunyor Jnr and Dr Paul Connell	48,755	
A/Prof Ian Trounce	44,800	
Prof Ian McAllister, A/Prof LRS Vijayasekaran, Prof Degli-Esposti and A/Prof Yu	49,995	
Dr Weiyong Shen and Dr Ling Zhu	50,000	
Dr Hannah Forward, Dr Charlotte McKnight and Dr Alexander Tan	50,000	
Dr Jelena Kezic	45,940	
	534,695	485,400
Deduct contribution from:		
Glaucoma Foundation Australia Inc	72,005	63,332
RANZCO Eye Foundation	120,000	100,350
	192,005	163,682
	342,690	321,718

	2012 $	2011 $
15 Funds Allocated to Director of Ophthalmic Research – Victoria		
Balance as at 1 July 2011	171,000	144,000
Interest for the year	2,530	1,408
Allocation for year	186,000	171,000
	359,530	316,408
Payment made to Director of Research	173,530	145,408
Balance as at 30 June 2012	186,000	171,000
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	(481,098)	394,473
– Administration	8,995	(5,031)
	(472,103)	389,442
Depreciation	689	90
Provision for Employee Benefits	3,908	2,300
(Increase)/Decrease in Receivables	208,586	(255,918)
Increase/(Decrease) in Creditors and Accrued Expenses	763	7,143
Increase/(Decrease) in Grants Payable	(85,302)	(93,306)
Increase/(Decrease) in allocation to Director of Research – Victoria	15,000	27,000
Valuation (Gains) on available-for-sale financal assets	301,269	(21,034)
(Profit)/Loss on Rearrangement of Investments	265,019	(170,354)
Net Cash Provided by/(used in) Operating Activities	237,830	(114,637)

18 Disclosures on Directors and other Key Management Personnel

Directors

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

A/Prof Robert Casson
Prof Stuart Graham
A/Prof Mark Daniell
Dr Richard Mills

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

Professor Stuart L Graham, Sydney (Chairman)
Professor Mark Gillies, Sydney (Vice Chairman)
Dr Richard Mills, Adelaide (Honorary Secretary)
Dr Wilson Heriot, Melbourne (Honorary Treasurer)
A/Prof Robert Casson, Adelaide
Dr Fred Chen, Perth
Dr Colin Clement, Sydney
Professor J Crowston, Melbourne
A/Prof Mark Daniell, Melbourne
Dr Paul Healey, Sydney
Dr Anthony Kwan, Brisbane
Professor David Mackey, Perth
Dr John Males, Sydney
Professor Peter J McCluskey, Sydney
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.

	2012 $	2011 $
(a) Short-term employee benefits	58,908	56,437
(b) Post-employment benefits	6,600	6,230
(c) Other long-term benefits	–	–
(d) Termination benefits	–	–
(e) Share-based payment	–	–
	65,508	62,667

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 2012.

(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 rates on investments.

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		1 to 5 years		Non Interest Bearing		Total Carrying Amount Per Balance Sheet	
	2012 %	2011 %	2012 $	2011 $	2012 $	2011 $	2012 $	2011 $	2012 $	2011 $	2012 $	2011 $
Financial Assets												
Cash and Cash Equivalents	3.50	4.50	957,903	860,199	–	–	–	–	–	–	957,903	860,199
Listed Investments												
Shares	N/A	N/A	–	–	–	–	–	–	7,391,966	7,754,214	7,391,966	7,754,214
Bank Bills	4.00	5,21	–	–	235,000	305,000	–	–	–	–	235,000	305,000
Receivables	–	–	–	–	–	–	–	–	144,665	353,250	144,665	353,250
Total Financial Assets			957,903	860,199	235,000	305,000	–	–	7,536,631	8,107,464	8,729.534	9,272,663
Financial Liabilities												
Payables	–	–	–	–	–	–	–	–	481,817	551,355	481,817	551,355
Total Financial Liabilities			–	–	–	–	–	–	481,817	551,355	481,817	551,355
Net Financial Assets			957,903	860,199	235,000	305,000	–	–	7,054,814	7,556,109	8,247,717	8,721,308

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 2012, 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
2012 Financial Assets					
Cash and Cash Equivalents	957,903	(9,579)	9,579	(9,759)	9,579
2011 Financial Assets					
Cash and Cash Equivalents	860,199	(8,602)	8,602	(8,602)	8,602

DIRECTORS' DECLARATION

The Directors of the company declare that:

1. The financial statements and notes as set out on pages 29–44:
   (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 2012 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.

Prof Stuart Graham, Director

Dr Wilson Heriot, Director

Sydney, this 22nd day of September, 2012

Orr, Martin & Waters
CHARTERED ACCOUNTANTS

461 Whitehorse Road
Balwyn
Australia 3103

Postal Address:
P.O. Box 307
Balwyn 3103

Partners:
John E Volders
Larry R Gilmour
Grant W Petering

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

Consultant:
Harold E Macmillan

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 2012, and the trust fund statement of comprehensive income, administration statement of comprehensive income, statement of changes in equity and statement of cash flows for the year then ended, notes comprising a summary of significant accounting policies and other explanatory information, and the directors' declaration.

Directors' Responsibility for the Financial Report

The directors of the company are responsible for the preparation of the financial report that gives a true and fair view in accordance with Australian Accounting Standards (including the Australian Accounting Interpretations) and the *Corporations Act 2001*, and for such internal control as the directors determine is necessary to enable the preparation of the financial report that is free from material misstatement, whether due to fraud or eror.

Auditor's Responsibility

Our responsibility is to express an opinion on the financial report based on our audit. We conducted our audit in accordance with Australian Auditing Standards. Those 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 report is free from material misstatement.

An audit involves performing procedures to obtain audit evidence about the amounts and disclosures in the financial report. The procedures selected depend on the auditor's judgment, including the assessment of the risks of material misstatement of the financial report, 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 report 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 report.

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, would be in the same terms if provided to the directors as at the date of this auditor's report.

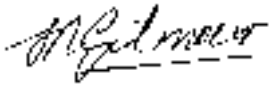
Auditor's Opinion

In our opinion the financial report of The Ophthalmic Research Institute of Australia is in accordance with the *Corporations Act 2001*, including:

(i) giving a true and fair view of the company's financial position as at 30 June 2012, and its performance for the year ended on that date; and

(ii) complying witht Australian Accounting Standards and the Corporations Regulations 2001.

Orr, Martin & Waters
Chartered Accountants



L.R. Gilmour, Partner
461 Whiehorse Road, Balwyn Vic 3103
25th day of September 2012.

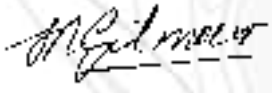
Auditor's Independence Declaration under Section 307C of the Corporations Act 2001

I declare that, to the best of my knowledge and belief, during the year ended 30 June 2012 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
461 Whiehorse Road, Balwyn Vic 3103
Dated this 25th day of Septembeer 2012.

NOTES

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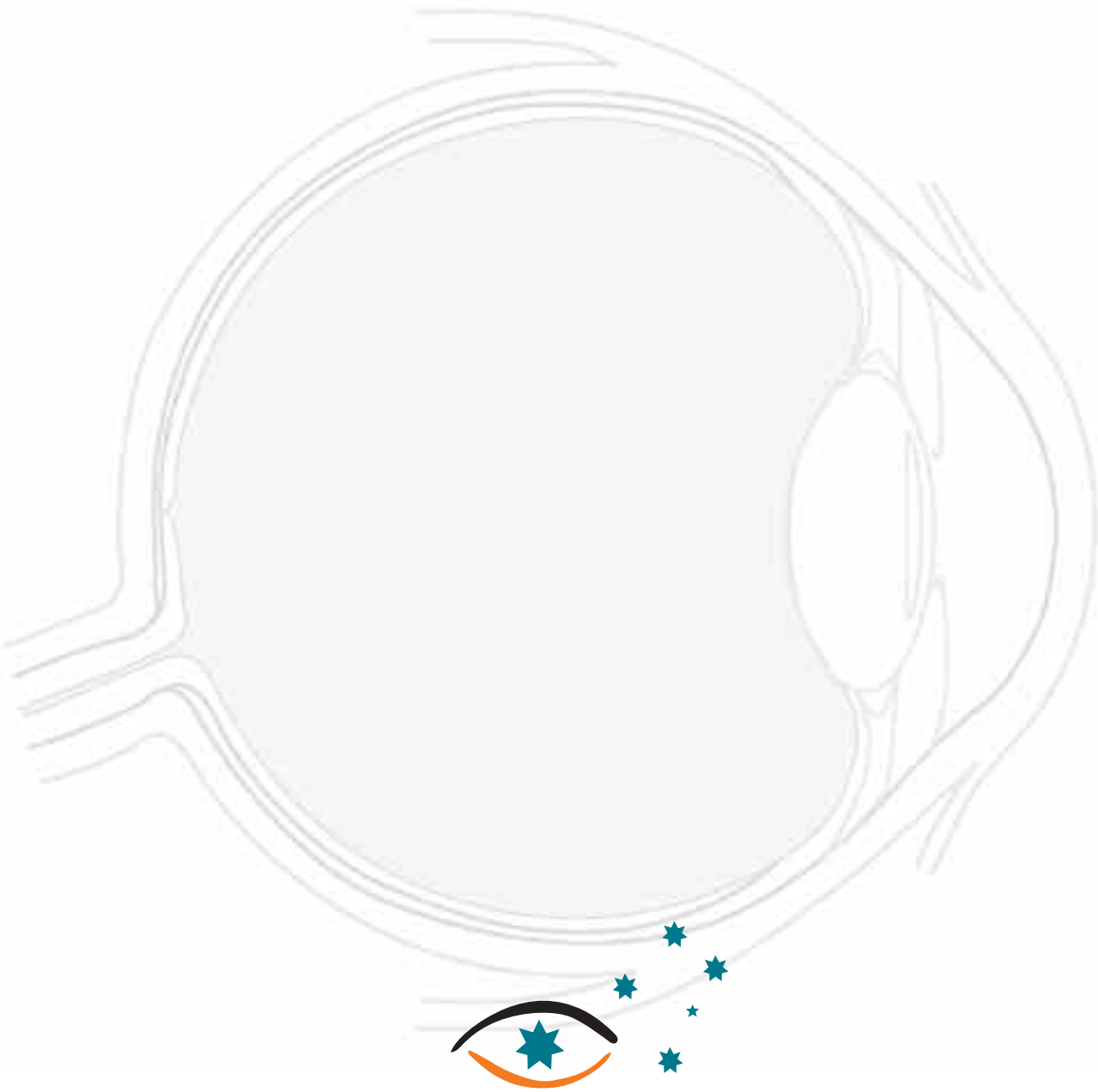
Printing: ABF Printers Pty Ltd, Arncliffe 2205



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