



## Introduction

Research at the Save Sight Institute focuses on the major causes of human blindness. From both clinical and laboratory perspectives the research groups aim to understand the world's major blinding conditions and to develop strategies to prevent them. The research groups work in close collaboration and are listed alphabetically.

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The Save Sight Institute

## Cataract and Presbyopia Research Group

### Group Members

Roger Truscott, BSc PhD, *Unit Head, NHMRC Senior Research Fellow*

Peter Hains, BSc PhD, *Postdoctoral Fellow*

Anastasia Korlimbinis, PhD, *Postdoctoral Fellow*

Eric Wei, MSc, *Research Fellow*

Michael Friedrich, *Postgraduate Student*

Karl Heys, *Postgraduate Student*

Jane Deeley, *Postgraduate Student*

### Research Activity

Research is focused on two conditions associated with aging: presbyopia (the inability to focus up close after middle age) and cataract (the major cause of world blindness).

On the basis of our research results, we have developed theories that can explain the origin of both of these debilitating visual conditions. It is proposed that nuclear cataract arises because of the age-related onset of a diffusion barrier within the lens. This 'barrier hypothesis' is now supported by a significant body of data. The end result of the barrier is protein oxidation in the lens nucleus. This arises because of a lack of sufficient antioxidants from the outer region reaching the centre of the lens. In addition, due to an increased residence time inside the barrier, there is an increase in the concentration of reactive species, derived from compounds such as UV filters. Together these factors lead to increased protein modification and nuclear opacification.

We believe that presbyopia is the result of a massive stiffening of the human lens nucleus. The centre of our lenses becomes harder by a factor of approximately 1000 fold over our life time. As a result, we can no longer change the shape of our lenses to focus on nearby objects after age 45-50. We are actively trying to understand the biochemistry responsible for this huge change in physical properties.

## Projects in 2005

### Posttranslational modification (PTM) in age-related cataract

Hains, Korlimbinis, Truscott.

The reason for the development of age-related nuclear cataract is still unclear. This blinding affliction is associated with major oxidation and colouration of the lens proteins. By working out the nature of these modifications we hope to identify the major PTMs that have brought about this change in the properties of the lens. In this way we may be able to understand what causes cataract. We employ mass spectrometry as one of a number of techniques to enable these alterations in protein structure to be elucidated.

### Ageing of human lens (presbyopia)

Heys, Freidrich, Truscott, Hains.

Human lens crystallins are present for the duration of a person's lifetime. In this period they become modified and as a consequence alter their properties. We have been monitoring some of these changes and relating them to alterations in the physical and optical properties of the lens. In this way we hope to understand one aspect of the biochemistry of ageing. Lenses were examined for stiffness and then were dissected into various regions and each extracted sequentially with buffer, 4M and 7M urea. Each fraction, including the membrane fraction, was analysed by SDS PAGE, bands quantified by scanning, and then each protein band identified by in-gel tryptic digestion followed by mass spectrometry of the peptides.

### Investigations into the lens barrier and its role in nuclear cataract

Berry, Friedrich, Truscott, Wei.

At middle age the lens nucleus becomes functionally uncoupled from the metabolically-active lens cortex. The resulting lack of adequate antioxidant defence renders the nucleus susceptible to oxidation. The existence of the lens barrier has been confirmed by NMR imaging. The consequences of the onset of the lens barrier are profound. It is not only the impediment to entry into the nucleus that is a problem; restricted exit from the lens centre also has deleterious consequences. The barrier hypothesis is increasingly recognised as the basis for understanding nuclear cataract. Ongoing studies into human lenses from the Lions Eye Bank were aimed at more precisely identifying the onset and the molecular basis of the barrier. This work involved the detailed proteomic analysis of the structure, function and interactions of molecules that play key roles in cell-cell communication.

### Analysis of lens membrane components as a function of age

Deeley, Truscott, Heys. Collaborators: Mitchell, Blanksby (University of Wollongong).

Over the life span of an individual, the lipid composition of the cell membranes in the lens changes substantially. The consequences of this are unknown, but we believe that such changes may also contribute to the development of presbyopia – the inability to focus on nearby objects after age 50. In continuing studies, we are characterizing cortical and nuclear changes in lens lipid composition and related these to separate measures of lens stiffness obtained by Dynamic Mechanical Analysis.

## Corneal Research Group

Corneal disease is a major cause of blindness worldwide. Research at the Institute aims to investigate the pathogenesis of the major blinding bacterial and viral corneal infections, improve the outcome of corneal transplants through development of corneal storage techniques and advancement of corneal surgery.

### Group Members

Kathy McClellan, MBBS PhD FRANZCO FRACS, *Unit Head and Medical Advisor Lions NSW Eye Bank*

Con Petsoglou, MBBS MMed (ClinEpi) FRANZCO FRACS, *Lecturer and Deputy Director Lions NSW Eye Bank*

John Males, MBBS BSc FRANZCO, *Associate Lecturer*

Li Wen, MBBS MMed, *Research Officer*

Raj Devasahayam, BAppSc, *Eye Bank Laboratory Manager*

Meidong Zhu, MMed PhD, *Eye Bank Laboratory Technician*

Mitchell Lawlor, MBBS, MMed(OphthSci), *PhD Student*

### Research Activity

A transparent cornea is vital to allow light to reach the retina. Further, the cornea provides the major refractive power of the eye for the focusing of images on the retina. Although the cornea and outer eye have a variety of well-developed protective mechanisms, the cornea remains vulnerable to infection. Blinding corneal disease may also be caused by developmental anomalies that result in progressive corneal opacity and distortion from birth.

Herpes simplex virus (HSV) infection occurs in up to 80% of the general population. HSV virus then remains latent in the human host and can recur in the form of cold sores or eye disease. HSV virus will cause eye disease in 1% of all people and may result in corneal

scarring and loss of vision. HSV is the commonest cause of infective unilateral vision loss. To better understand this virus and prevent this cause of vision loss we are investigating its distribution and latency in trigeminal ganglia and the cornea as well as its role in suppurative keratitis.

Bacterial corneal infection or bacterial keratitis is the most important diagnosis in the patient with a red eye. Every year approximately 200 patients are admitted to the Sydney Eye Hospital with this condition. To understand the trends in this blinding disease we have determined the antibiotic susceptibilities of bacterial ocular isolates to better guide the emergency management of red eye. This information allows effective and rational antibiotic therapy of corneal infection.

The Lions NSW Eye Bank is situated in the Save Sight Institute and provided 510 corneal grafts to the patients of NSW in 2005. Recent research by our PhD fellow has resulted in the implementation of scheduled surgery for the patients in NSW. This has seen a dramatic reduction in the waiting time for corneal transplantation to an current average of 9 months. The laboratory staff of the Eye Bank are also investigating methods of corneal storage that extend the life of corneal transplants.

## Projects in 2005

### The 22nd Cornea and Eye Bank Meeting

The Corneal Research Unit had the opportunity of hosting this annual conference in February 2005. This meeting marked the 100th year since the first successful cornea transplant in Czechoslovakia in 1905. The meeting was attended by 85 Australian, New Zealand and international cornea specialists and Eye Bank staff. It provided an excellent opportunity for the discussion of present research into corneal diseases and eye banking. Opened by the NSW Chief Medical Officer, Dr Denise Robinson, there was significant discussion on the role of research institutes in the improvement of health outcomes.

### Human herpes simplex virus (HSV) and herpes zoster virus

McClellan, Petsoglou, Wen.

The group is gaining significant expertise in the techniques of PCR analysis (real time and in-situ) and immunohistochemistry for the human herpes virus. Ongoing projects include the distribution and form of these viruses in healthy human trigeminal ganglia and corresponding corneas, the genotyping of herpes viruses to identify virulent strains, identification of their prevalence in suppurative keratitis, their role in corneal graft failure without rejection and detection in iridocorneal endothelial syndrome.

### Antibiotic susceptibilities

McClellan, Males, Li.

Antibiotic susceptibilities of corneal isolates and conjunctival flora have been determined in 125 organisms isolated from 80 patients presenting to the Sydney Eye Hospital Emergency Department during the past 12 months. These have been published and continue to guide the emergency management of bacterial keratitis.

### Corneal storage

Devasayaham, Zhu, McClellan.

The development of an optimised organ culture medium for corneal grafts is being actively researched. This involves the investigation of corneal metabolism during storage and the viability of endothelial cells after transplantation.

### Infectious keratitis

McClellan, Males.

Infectious keratitis is being studied in 3 groups of patients - contact lens wearers, the elderly and patients with corneal grafts - to determine risk factors, common pathogens and outcomes, with the aim of better preventing and managing this condition.



Fungal keratitis

### Pterygium

McClellan, Males, Petsoglou.

In recurrent pterygium the group has had extensive experience in the use of lamellar keratoplasty for the surgical correction of pterygium. The outcomes of the operation were evaluated and published.

### Corneal transplant in infants and children

McClellan.

This study was the first to demonstrate graft survival beyond 14 years amongst this group of young recipients. We are continuing to investigate the cause of visual impairment in this group of patients.

### Corneal confocal microscope

Devasayaham, McClellan, Petsoglou.

A corneal confocal microscope was acquired through the Sydney Eye Hospital Foundation. Research into the use of the microscope in the diagnosis, investigation and treatment of corneal diseases is being undertaken. Specific expertise and research into acanthamoeba and fungal keratitis has led to results being presented at conferences.

### Digital Media Research Group

Research in this group is focused on exploiting the potential of digital media, information technology (IT) and the communications revolution to:

- Create and implement state of the art outreach ophthalmic services for remote and rural communities.
- Develop cutting edge ophthalmic teaching services to provide excellence in education of future eye doctors.
- Empower the community via the internet by providing interactive access to information about eye care and lifestyle choices which can help prevent eye disease.

### Group Members

Frank Billson, MBBS FRANZCO FRACS FAC  
FRCOphth, *Unit Head*  
I-van Ho, *Postgraduate Student*

### Research Activities

**Teleophthalmology Initiative:** The challenge of providing specialist eye care services outside the metropolitan centres of Australia is a significant one. This group is working to develop more effective ways of screening for eye disease in remote and rural areas.

**Virtual Teaching Initiatives:** The unit is researching initiatives to provide distance learning services and developing innovative ways for students to have clinical trial experience in a virtual arena before engaging in patient practice.

**Interactive Community Services:** As digital media increasingly become a delivery tool for community information and access to services, this group is focusing on improving ways of delivering sight saving information to the community and developing simple interactive eye services to be accessed via e-media

### Projects in 2005

#### Virtual Ophthalmology Clinic

The unit's virtual ophthalmology programme allows medical students to gain skills in history taking by making use of "virtual" clinical patients. The histories in the programme's database, transcribed from real patient interviews and presented by interactive virtual actors, are programmed to reflect symptoms associated with common eye diseases and complaints.

The programme mimics real interactions with patients in that students control the order in which they ask questions and gather information to make a diagnosis prior to an examination. The diagnosis is emailed to the student's supervisors before the student is allowed to continue the examination and higher levels of investigation. The strength of the programme is that it allows the student to develop skills in interviewing and forming a diagnosis before practising on real patients. Its success as a teaching and self-development tool highlights the direction of future digital and distance learning initiatives.

The programme is now deliverable via the internet. Recently the interface was partly redesigned for improved navigability and its networking capabilities were fine-tuned to enhance the programme's utility across a wider range of environments. It is also envisaged that it be included in the course content for the Graduate Diploma and Masters Degree in Ophthalmic Science, a distance and onsite learning collaboration between the University of Sydney and the University of Otago, which began in 2004.

#### Tele-ophthalmic diabetic eye screening and treatment programme

The tele-ophthalmology initiative in the Northern Territory continued in 2005. There has been a significant increase in the number of remote Aboriginal communities successfully screened for diabetic retinopathy.

A similar study is underway in the Macquarie Area Health region in NSW.

A fully equipped facility has been developed in the Dubbo Aboriginal Medical Coperative Centre. A skill-transfer programme will continue and the data generated will form an important opportunity in the development of a database for analysis, working with the Aboriginal Centre to develop strategies that will improve access to and understanding of eye health among Aboriginal people. It will be possible to integrate the service, with Aboriginal agreement, with the Dubbo Rural Clinical School.

## Electrophysiology and Glaucoma Research Group

### Group Members

Alex Klistorner, BMed PhD  
Stuart Graham, PhD MBBS MS FRANZCO FRACS  
John Grigg, MBBS FRANZCO FRACS  
Frank Billson, MBBS FRANZCO FRACS FAC  
FRCOphth  
Clare Fraser, MBBS, *Postgraduate Student*  
Alessandra Martins, MBBS, *Postgraduate Student*  
Chandra Balachandran, MBBS, *Postgraduate Student*  
Hemamalini Srinivasan, *Glaucoma Clinical Fellow,*  
*Sydney Eye Hospital*  
Lisa Feldman, DipNursing, *Electrophysiology*  
*Technician*  
Asya Klistorner, *Electrophysiology Technician*  
Dr Maria Kosokova, *Electrophysiology Technician*

### Research Activity

The electrophysiology section continues to provide clinical testing as well as research into new techniques for electrodiagnosis, especially for the detection of glaucoma. In 2005 the department acquired a new electrodiagnostic system, the Espion Diagnosys system. The Espion system was designed to easily perform all the current standard clinical visual function tests, including ERG, VEP, PERG, EOG, Flash-VEP and Pattern VEP. The Espion Diagnosys System comes programmed with the ISCEV standards as well as allowing modifications for research. It also has a hand-held mini ganzfeld which is useful for testing children. It will also enable testing of children without the need for sedation. Following acquisition of a normative database for the tests from the local population the system has provided a more reliable and sensitive system for detailed assessment of patient's visual function.

### Projects in 2005

#### Development of Objective Perimetry – the Multifocal Visual Evoked Potential

Klistorner, Graham, Grigg, Billson.

The Save Sight Institute continues to work in collaboration with ObjectiVision Pty Ltd to develop the AccuMap V2 objective perimeter as a method for investigating visual function particularly in glaucoma. The AccuMap V1.3 was successfully launched in Australia, but despite FDA approval was not widely adopted in the USA. We have therefore sought to address some of the limitations of the system and improve its diagnostic performance to make it a potential benchmark for objective detection of glaucoma. The version 2 system is in the final prototype phases, and will incorporate several major advances. Firstly, the original large CRT screen is replaced with

a flat high speed LCD screen, and the computer is replaced with a laptop, both making the system much more compact and potentially portable. Secondly, a completely new electrode headset has been designed with a preamplifier located on the headset itself. This dramatically improves signal to noise ratios and will improve reliability of recording and shorten test times. Thirdly, a new disposable electrode cross has been developed which can be clipped onto the preamplifier for recording then discarded. This saves time for the clinician and removes hygiene issues associated with cleaning and re-use of the electrodes. The new system will also incorporate new software for calculating the signal latency which is very important in optic neuritis (see project p6).

#### Development of a Binocular Multifocal Visual Evoked Potential using Virtual Reality Goggles

Klistorner, Graham, Srinivasan, Grigg.

We have received an NHMRC Development Grant for the extension of the mVEP technique to use virtual reality goggles as a means of presenting the stimulus. This has the potential advantages of making the test portable and standardising the test distance. It also allows for the testing of both eyes at the same time. This has the advantage of allowing intereye comparisons to look for subtle early changes. Our pilot study has shown for the first time that not only is it possible to record from both eyes simultaneously, but that the technique definitely works in glaucoma, with 10 consecutive cases correctly identified. The research will now attempt to optimise the stimulus design and signal extraction with a view to conducting a larger clinical trial.

In 2001 ObjectiVision Pty Ltd was set up as a collaboration between investigators at the Save Sight Institute and the Sydney University Business Liaison Office to develop new techniques in vision testing. In 2002 the AccuMap V1.3 objective perimeter was launched as a new test for glaucoma that relied not upon the patient's subjective responses but on recording the tiny electrical signals generated in the brain, called visual evoked potentials, when the patient was viewing a stimulus on a computer screen. The initial AccuMap won 2 Australian design awards in 2002.

The AccuMap then was redesigned with a major software upgrade using the OPERA V2.0 platform. This was developed to include new noise detection parameters and make the interface much more user-friendly. The system received FDA approval late 2003, and was launched in the USA at the American Academy of Ophthalmology. Heidelberg Technologies have been contracted as US distributors.

At present there are 5 US trial sites involved in the multicentre AccuMap Early Glaucoma Detection Study, with Dr Klistorner and Dr Graham at the Save Sight

Institute being co-ordinators. The results of this study will help confirm the effectiveness of the technique compared to conventional methods of detecting glaucoma. Dr Grigg and Prof Billson are principle investigators.

We have recently been awarded an NHMRC Development Grant for the extension of the technique to use virtual reality goggles as a means of presenting the stimulus. This will have the potential advantage of making the test portable and standardising the test distance.

#### **Development of a Blue/Yellow Multifocal Visual Evoked Potential**

Martins, Klistorner, Graham, Balachandran.

This research investigates the use of blue-yellow stimuli for presenting the multifocal Visual Evoked Potential (mVEP) in an attempt to detect glaucoma at an earlier stage. The rationale for this is that in subjective perimetry blue/yellow testing has been shown to be more effective at detecting early disease. To date, the mVEP technique has used a black and white stimulus to demonstrate abnormal visual field defects in glaucoma cases. This research group, in collaboration with Dr A James from ANU Canberra, has now developed an optimal design which maximises the mVEP amplitudes for the stimulus. The blue/yellow alternating checkerboard with isoluminantly matched checks has now been incorporated in the AccuMap system. This work was supported by funding from an ORIA grant in 2004 and 2005.

The test has been applied to normal subjects in order to create a normal blue-yellow database and a study has just been completed of 50 early glaucomas and 60 normals. The results of this study show that based on amplitude of the response the new B onY mfVEP can detect early glaucomatous visual field defects with a high degree of accuracy. In addition, our study indicated that visual field defects were more extensive when blue color stimulation was used compared to achromatic stimulation. It was particularly evident in eyes with minimal changes on B/W mfVEP. This is comparable with subjective visual fields, where significantly more extensive local and diffuse loss of sensitivity was found on SWAP perimetry compared to achromatic stimulation and this was especially prominent in early glaucoma. There were some abnormal B onY mfVEP results identified in perimetrically normal areas of glaucomatous eyes and in some fellow eyes, which may indicate an ability of the technique to detect loss earlier than subjective testing. Further studies will confirm the ability of this test to predict early damage in glaucoma. Currently, the technique is being applied to established glaucoma patients and high risk suspects for glaucoma to establish its sensitivity in comparison with other diagnostic techniques such as

Humphrey Visual Fields-the gold standard, black-white mVEP, Short-Wave Automated Perimetry and Optical Coherence Tomography.

#### **Multifocal Visual Evoked Potentials in Optic Neuritis**

Fraser, Grigg, Klistorner, Graham, Garrick, Billson.

Multiple Sclerosis (MS) can damage the optic nerve leading to vision loss, this can be the first presentation of MS in 30% of patients. The resulting optic neuritis causes the loss of the protective layer surrounding the nerve fibres. Consequently there is a disruption in the electrical signal transmitted from the eye to the brain. Visual Evoked Potentials (VEPs) record the electrical responses in the brain that represent the transmission of this visual information from the eye.

Current VEP recordings only measure these responses from the central visual field and cannot differentiate non-specific exacerbations of optic neuritis from true recurrences. A pilot study conducted at the Save Sight Institute has shown that the multifocal VEP (mVEP), measured by AccuMap, can localise optic neuritis abnormalities to a specific visual field area and thus potentially demonstrate exacerbations of existing lesions versus new demyelination. A 12 month study of optic neuritis conducted by Dr Clare Fraser *et al* showed that significant signal delays seen in optic neuritis were predictive of the subsequent development of MS. This could have great clinical significance in terms of patient monitoring, treatment and prognosis. We are continuing the testing of a prospective study in collaboration with the Melbourne Eye and Ear Hospital and Auckland University using the AccuMap mVEP system of objective perimetry to detect changes associated with optic neuritis. The AccuMap will provide an objective measure of a patient's visual field, as well as information on the axonal function between the retina and the occipital visual cortex. We hope to use the AccuMap to detect subtle and more peripheral vision changes than conventional VEP and provide the first detailed means to monitor recovery of nerve function. By studying the changes in mVEPs seen in acute optic neuritis and then following these changes over time we hope to develop the mVEP as a useful tool for monitoring the effects of the new treatments for MS on recovery of visual function and as a marker for remyelination. Old lesions can be identified, but a chronic inflammatory change (potentially reversible) in the optic nerve may be also be identified if it can be seen to resolve with new forms of treatment.

We also hope to detect sub-clinical remissions or new lesions within the visual pathways. This may allow more patients earlier access to interferon therapy. Furthermore the mVEP could be a sensitive tool to detect remyelination, and if this could be achieved, help evaluate future therapies.

## Education

The electrophysiology group has supervised two PhD students and one Masters student during the year with one PhD student and the Masters student submitting their theses. The unit staff have been prominent in scientific meetings.

Dr Graham was a member of the scientific programme committee for the Asian-Oceanic Glaucoma Society (AOGS) congress held in Cairns in September 2005. In July Dr Graham presented at the World Glaucoma congress an update on objective perimetry.

Dr Fraser was awarded the best student prize for her presentation at the November 2004 Neurophthalmology Society of Australia annual meeting. The prize was a funded trip to the North American Neurophthalmology Society meeting where she again won the prize for best student presentation for her work on optic neuritis and latency as assessed by the Accumap.

Dr Grigg contributed to the organisation of a mini symposium on paediatric glaucoma at AOGS. Dr Grigg presented "A clinical approach to electrophysiology" at the NSW RANZCO Branch meeting held in April 2005.

## Eye Genetics Research Group

### Group Members

Robyn Jamieson, MBBS FRACP PhD, *Unit Head*

John Grigg, MBBS FRANZCO FRACS

Frank Billson, MBBS FRANZCO FRACS FAC  
FRCOphth

Chris Willcock, BSc(Hons), *Research Assistant*

Marija Mihelec, BSc(Hons), *Postgraduate Student  
(CMRI)*

Luke St Heaps, BSc(Hons), *P/T Postgraduate Student  
(CHW)*

### Research Activity

Genetic eye disorders contribute to the causes of blindness and partial-sightedness for many with visual disability in our community. Our research studies in genetic eye disease focus on developmental ocular conditions including cataracts (clouding of the lens), glaucoma (raised pressure in the eye and optic nerve abnormality), retinal anomalies (disorders affecting the back of the eye) and microphthalmia or anophthalmia (small or absent eye). All of these conditions can lead to visual disability or blindness and in all there are few or limited treatment options. Treatment is

difficult both for initial management and also in the prevention of ongoing vision loss for the child as he or she grows and develops. Current treatments are often surgically orientated, and while such treatments may still be required, this research work aims to discover disease genes important in these conditions, and the functions of the proteins they encode. By understanding the detailed protein functions, we will then be able to develop better medical treatments to vastly improve the management of these conditions.

In this research programme we are studying patients and families who have developmental eye conditions, who also have clues on their history or assessment to provide an entry point through which we can work to identify the underlying disease gene. These include families which are large and suitable for linkage analysis and families where there are chromosome changes which are leading us to the underlying disease gene. We examine other patients and families seen through the Save Sight Institute for changes in these genes. We also study the mouse as a model to understand the detail of the functions of disease genes which cause abnormalities of eye development. We have collaborative links with the Children's Medical Research Institute (CMRI), the Western Sydney Genetics Programme and the Discipline of Paediatrics and Child Health at the Children's Hospital at Westmead (CHW).

### Education

Dr Robyn Jamieson was an invited speaker at the Conjoint Meeting of the Asian Oceanic Glaucoma Society and ANZ Glaucoma Club meeting in Cairns in September 2005 and was also invited to speak on Ophthalmic Genetics at the Royal Australian and New Zealand College of Ophthalmologists, NSW Branch meeting in April 2005.

Ms Marija Mihelec received the research student prize at the Human Genetics Society of Australasia, NSW Branch Meeting, in May 2005 for her presentation "Ocular developmental anomalies and translocation breakpoint mapping".

Ms Renata Krowka, medical student, University of Sydney, received the Robert Scot Skirving Memorial Prize for the Best Final Year Student Elective for her elective project conducted in the Eye Genetics Research Group "Genes in Eye Development".

Dr Jamieson contributed to organisation of the scientific programme of the annual meeting of the Human Genetics Society of Australasia held in Newcastle in July 2005.

## Lens Research Group (Cellular and Developmental Biology)

Research is directed at identifying molecules and mechanisms that govern the behaviour of lens cells in health and disease. Our studies have identified molecules that play key roles in both normal and pathological lens development. Currently we are working to gain a better understanding of how these molecules are regulated in the eye. This is fundamental to identifying new therapeutics for retarding or preventing cataract, one of the most common (and costly) diseases of ageing.

### Group Members

John McAvoy, BSc PhD, *Unit Co-Head*  
Frank Lovicu, BSc PhD, *Unit Co-Head*  
Richard Stump, BSc PhD, *Laboratory Manager*  
Jessica Boros, BSc, *Research Assistant*  
Sharon Ang, BSc, *Research Assistant*  
Iaxmi Iyengar, BSc, *Postgraduate Student*  
Yongjuan Chen, BSc, *Postgraduate Student*  
Colin Chong, MBBS, *Postgraduate Student*  
Kevin Wang, BSc, *Postgraduate Student*  
Bramilla Patkunathan, BSc, *Postgraduate Student*  
Grace Lam, BSc MSc, *Postgraduate Student*  
Peter Newitt, BSc, *Postgraduate Student*

### Research Activity

The lens transmits and focuses light onto the retina. To do this it needs to be transparent and to have appropriate refractive properties. This depends on the development and maintenance of highly ordered cellular architecture.

The lens consists of two forms of cells encapsulated within a basement membrane; (i) elongated fibre cells grow to several millimetres in length and are precisely aligned to form a regularly packed spheroidal mass and (ii) cuboidal epithelial cells form a single-layered sheet that covers the anterior surface of the fibres.

Whilst the fibre cells make up the bulk of the lens and mostly determine its optical properties, epithelial cells play a key role in maintaining an appropriate physiological environment within the lens. In addition, the epithelium contains the 'stem cells' that proliferate, migrate and differentiate into the new fibres that are progressively added to the fibre mass throughout life.

We have focused our attention on growth factors because of their importance in regulating cell fates in developmental systems. Using a unique lens epithelial explant culture system we have identified members of the FGF growth factor family as inducers of lens cell

proliferation, migration and differentiation; responses that are induced in a progressive dose-dependent manner. We have proposed that an anterior-posterior gradient of FGF determines lens polarity and growth patterns. A major thrust of research activity in our laboratory is aimed at testing this hypothesis.

Insights into the molecular basis of the major lens pathology, cataract, have also arisen from our growth factor studies. We have shown that members of the transforming growth factor beta (TGF $\beta$ ) family induce aberrant growth and differentiation of lens cells. This progressively leads to disruption of normal cellular architecture and opacification of the lens.

Cataract is the most common cause of blindness in the world today. Although surgery is generally effective, in many countries it cannot keep pace with the growing demand. Moreover, complications such as aberrant growth and differentiation of lens cells left behind after cataract surgery (most commonly referred to as posterior capsule opacification), require further treatment and add to the cost of cataract management. Because of its clinical significance it is vital to understand how TGF $\beta$  is regulated in the eye and how it induces cataractous effects on the lens. This information is fundamental to understanding the molecular basis of cataract and devising strategies for prevention.

### Projects in 2005

#### The role of FGF antagonists in lens biology and pathology

Newitt, Boros, McAvoy, Lovicu.

The levels of growth factors found in the eye, such as FGF, need to be tightly regulated as they are important for the maintenance of lens growth and architecture. Any disruptions to the levels of growth factors in the eye will readily disrupt lens cell behaviour, leading to loss of lens transparency and development of cataract. To better understand how FGF signalling is regulated in different compartments of the eye, we set out to identify the distribution of members of the Sprouty and Sef gene families that have recently been reported to be FGF antagonists. We showed that their distribution was consistent with a role in inhibiting fibre differentiation and maintaining the epithelial phenotype in the anterior segment. We plan to test the model that these FGF antagonists are important for maintenance of the normal lens polarity (i.e epithelial cells anteriorly and fibre cells posteriorly), and that disturbances in their expression (by specifically overexpressing them in the lens of transgenic mice or functionally deleting them from the lens) can lead to disturbances in lens development, potentially leading to cataract.



### **FGF signalling and lens cell proliferation and differentiation**

Lovicu, Iyengar, Wang.

These studies were aimed at elucidating how FGF induces different cellular responses. We investigated the intracellular signalling cascades that are activated by FGF. We showed that the ERK1/2 (MAPK1/2) signalling cascade is involved in both proliferation and differentiation. Inhibition of ERK1/2 blocks cell proliferation; however, it blocks only the morphological (elongation), not the molecular (beta-and gamma-crystallin gene expression) aspects of differentiation. This indicates that fibre differentiation depends on activation of several intracellular signalling cascades. The dynamics of these growth factor-induced cascades were also studied in relation to the signalling cascades induced by the ocular media.

### **PDGF/IGF/EGF signalling and lens cell proliferation**

Lovicu, Iyengar, Wang, Patkunathan.

IGF/insulin and PDGF growth factor families can induce lens epithelial cells to undergo cell division. To determine if IGF and PDGF utilise the same signalling pathway(s) as FGF, we examined the role of ERK1/2 signalling in IGF- and PDGF-induced lens cell proliferation. Similar to FGF, both IGF and PDGF can induce lens cell proliferation, in an ERK-dependent fashion. However, PDGF activates ERK within 10 minutes compared to 20 minutes for IGF. Differences in the temporal activation of lens cell proliferation by IGF and PDGF may be due to differences in the mode by which these growth factors activate ERK1/2. The dynamics of these growth factor-induced cascades were studied in relation to the signaling cascades induced by the ocular media. Overall, these results support an important role for ERK signalling in growth factor-induced lens cell proliferation.

### **Lens regeneration**

O'Connor, Lovicu, McAvoy.

FGF is good at inducing fibre differentiation in lens epithelial explants. However, elongating cells in FGF-treated explants do not show the same degree of alignment with their neighbours as do elongating fibres in vivo. There is growing evidence to suggest that other, as yet unidentified, factors are involved in coordinating the differentiation of the epithelial 'stem cells' and their elongation into highly aligned fibre cells. Recent developments in tissue culture techniques pioneered by our laboratory are enabling a more rigorous investigation of the intraocular fluids in search of these factors. This raises the possibility of devising strategies that will promote lens reconstruction after cataract surgery.

### **Crim 1 expression and function in the lens**

Lovicu, Boros, Little (University of Queensland), McAvoy.

We localised a new gene, Crim1 (initially identified by colleagues at University of Queensland), during eye morphogenesis. Crim1 is particularly strongly expressed in the lens epithelium. During postnatal growth, expression is reduced in all ocular tissues and eventually it is only expressed in the lens. Investigating the functional significance of Crim1 in the lens in gain-of-function and loss-of-function mouse models forms the basis of an ongoing collaborative study with colleagues at the University of Queensland.

### **Wnt signalling in lens development**

Chen, Stump, Ang, Lovicu, McAvoy.

We studied the expression of members of the Wnt growth factor family and its receptors, the frizzleds (Fzs). Wnts are commonly involved in regulating expression of molecules involved in cell proliferation, communication and adhesion. We showed that multiple Wnts and Fzs are expressed in the lens. In addition, we showed that key Wnt/Fz signalling molecules, including those involved in Wnt/ $\beta$ -catenin and Planar Cell Polarity pathways, are expressed in the lens. A mouse mutant that is deficient in a co-factor required for Wnt/ $\beta$ -catenin signalling does not develop a complete lens epithelium. In addition, a transgenic mouse that overexpresses an inhibitor that blocks all Wnt signalling pathways, secreted frizzled-related protein 2, shows severe inhibition of fibre differentiation and develops cataract. These results support our hypothesis that several Wnt signalling pathways play key roles in the differentiation/maintenance of both forms of lens cells.

### **Wnt signalling in TGF $\beta$ -induced cataract**

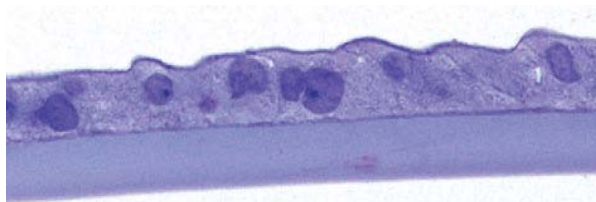
Chong, Ang, Lovicu, McAvoy.

Transforming growth factor-beta (TGF $\beta$ ) induces aberrant growth and differentiation in rodent lenses that is characteristic of some forms of human cataract. As changes in Wnt signalling are frequent key events in abnormal growth and differentiation in other systems, we investigated Wnt expression in established TGF $\beta$ -induced cataract models. We showed that Wnt expression is altered in TGF $\beta$ -induced rat and mouse cataract models. Levels of Wnt5a and Wnt7b protein and mRNA are distinctly upregulated in cataractous plaques of all the models we examined. This suggests that de-regulation of Wnt signalling is involved in regulating abnormal growth and differentiation processes in TGF $\beta$ -induced cataracts and may play a role in human cataract, such as posterior capsule opacification.

### **Lithium stabilises the polarised lens epithelial phenotype and blocks TGF $\beta$ -induced cataract**

Stump, Ang, Lovicu, Pandey (Intraocular Implant Unit, Sydney Eye Hospital), McAvoy.

A common complication of cataract surgery is caused by epithelial mesenchymal transition (EMT) and aberrant growth of residual lens cells. As recent studies indicate a role for Wnt/ $\beta$ -catenin signalling in lens epithelial differentiation, we assessed the effects of lithium chloride (LiCl), which is often used to mimic aspects of  $\beta$ -catenin signalling, on regulating the behaviour of cells in lens epithelial explants. In controls, cells depolarised, proliferated and migrated. In contrast, in LiCl-treated explants cells remained in discrete groups, did not proliferate or migrate and maintained their normal polarity and cobblestone-like packing. In controls,  $\beta$ -catenin was initially localised to the cell margins but as cells depolarised and migrated, it became distributed throughout the cytoplasm and was particularly strong in nuclei. Many of these cells also showed activation of the TOP-green reporter indicating canonical  $\beta$ -catenin signalling. By contrast, in polarised LiCl-treated cells,  $\beta$ -catenin remained localised to cell margins and, as in vivo, there was no evidence of  $\beta$ -catenin signalling. Significantly, the effects of lithium also extended to blocking the cataract-promoting effects of TGF $\beta$  on both rat and human explants. This opens up possibilities for developing molecular approaches for maintaining the polarised epithelial phenotype and preventing aberrant growth that leads to cataract.



Lithium treated lens cells

### **Vitronectin in lens development and cataract**

Ang, Taliana (Invitrogen Corporation), Evans (CSIRO, West Ryde, NSW), McAvoy.

The lens capsule forms during lens morphogenesis and provides the substratum for lens cell attachment, growth and differentiation. Its primary extracellular matrix (ECM) constituents include type IV collagen, laminin, heparan sulphate proteoglycan, fibronectin and entactin/nidogen. Recent studies have shown that during the aberrant growth and differentiation seen in some forms of cataract, abnormal accumulations of ECM are deposited as lens cells undergo epithelial mesenchymal transition. One of the molecules identified within the resulting fibrotic plaques is vitronectin (Vn). We showed that Vn is expressed by lens epithelial cells and is present in lens capsule during development.

Lens epithelial cells can attach and migrate on Vn; however, on this substratum, the cells undergo an epithelial-mesenchymal transition that is characteristic of cataract. This result raises questions as to how, or if, lens cells and Vn interact in normal development and how this relationship may be disrupted during cataract formation.

## **Retinal Research Groups**

The retina, which detects light like the film of a camera, is an exquisitely complex and delicate part of the eye. Retinal diseases, such as diabetic retinopathy and age-related macular degeneration, are now the commonest causes of untreatable blindness in Australia.

The SSI has three research groups working on various aspects of retinal biology and pathology:

- 1) Retinal Cell Death and Survival
- 2) Retinal Development, Ageing and Eye Cancer
- 3) Retinal Therapeutics

The groups work in close association – sharing information, insights and frequently joint publications.

Age-related macular degeneration is a common thread of research in terms of:

- Basic biology, development and changes in associated vasculature.
- The importance of therapy for sub-retinal new vessels in the blinding stage of this disease.
- The fact that genetic factors may be important in susceptibility to the disease.

## **Retinal Cell Death and Survival Research Group**

This group, now located in the Research School of Biological Sciences at the Australian National University, has been associated with the Save Sight Institute since 2002. The group has several collaborative projects with Save Sight Institute researchers and a growing track record of joint publications.

### **Group Members**

Jonathan Stone, BSc PhD FAA, *SSI Associate (ANU), Unit Head*  
Diana van Driel, BSc(Hons), *Senior Research Assistant*

### **Research Activity**

Research concerns the mechanisms which control the death and survival of neurones in degenerative retinal disease. The group has made a series of findings

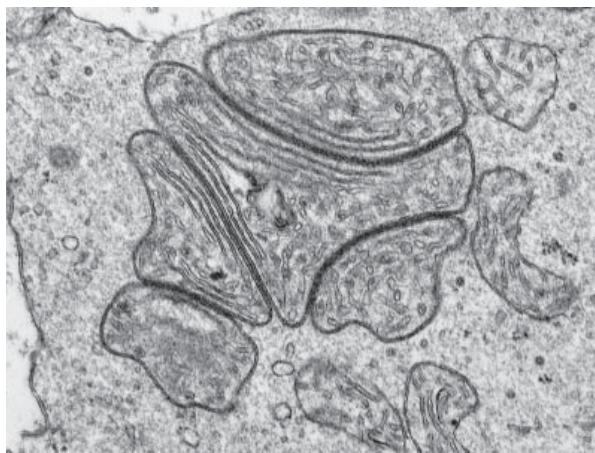
novel in the understanding of photoreceptor death and survival in a range of rodent models and in the human.

New techniques and collaborations have been developed to take the analysis of the mechanisms of photoreceptor disease to the molecular level and to include the epidemiology of human disease.

Clinical trials have been organised to deploy some of this knowledge.

### Projects in 2005

Current joint projects include the examination of mitochondrial specialisations in the normal and degenerative retina, and clinical trials of light restriction.



Photoreceptor synapse

## Retinal Development, Ageing and Eye Cancer Research Group

Research aims to shed light in two areas - Retinal Development and Eye Cancers - and to explore the inter-relationships between them.

### Group Members

Michele Madigan, BOptom PhD, *Unit Co-Head*  
Max Conway, PhD FRACO FRACS, *Unit Co-Head*  
Jan Provis, BSc PhD, *SSI Associate (ANU)*  
Diana van Driel, BSc(Hons), *Senior Research Assistant*  
Elisa E Cornish, BMedSci PhD, *Research Assistant (P/T)*  
Alexandra Allende, MBBS, *Postgraduate Student*  
Trong Van Pham, MD, *Postgraduate Student*  
Kenneth Lai, BMedSci, *Postgraduate Student*

### Member advising on clinical aspects

Meidong Zhu, MD PhD, *Research Fellow*

## Research Activity

The human 'macula' is a region of the retina that includes several specialisations not present in non-primate retina and which together enable us to see detail. Understanding the development and ageing of the macula and how this unique structure makes humans vulnerable to degenerative disease is the principle aim of our research into development and ageing. The fovea is at the geometric centre of the macula and is identifiable by a number of structural modifications including: (1) an absence of a direct blood supply from the retinal circulation, (2) the presence of a pit which thins the retina locally, (3) a focal concentration of a class of colour sensitive photoreceptors (cones) and (4) the inclusion of specialised circuitry which conserves the electrical responses of individual cones. Few groups internationally are applying modern molecular tools to the investigation of primate retinal development and human retinal disease. We have developed new approaches to investigate the biology of the macula, our focus being to identify the unique molecular profiles associated with the unique anatomy and physiology of the macula and fovea. Better understanding the normal processes of development and ageing in the retina and choroid also provides insight into the pathogenesis of primary eye tumours. In particular, we are studying retinoblastoma (Rb) (derived from retinal neuroblasts) and ocular melanoma (affecting choroid, ciliary body or iris); these are the most common primary intraocular eye cancers in children and adults respectively. We are investigating the mechanisms controlling cell proliferation, cell death and tumour invasion and angiogenesis, in order to better understand the pathogenesis of these tumours. Combined clinical and laboratory research provides an understanding of the biology of these tumours and may improve the rationale for treatment. This is especially important given the morbidity associated with enucleation, the side effects of current therapies, particularly radiation, and the high incidence of untreatable metastases in ocular melanoma.

### Projects in 2005

#### Growth factors in retinal development

Allende, Cornish, Natoli (ANU), Madigan, Provis (ANU).

Fibroblast growth factors (FGFs) regulate a number of cellular functions including morphological differentiation (ie changes in cell shape). At the fovea – the part of the retina that enables us to discriminate fine detail – cones are highly elongated and very narrow, enabling them to pack at high density. During ageing and in macular degeneration this morphological specialisation is lost and is associated with a decline in visual acuity. Our studies show that during development members of the FGF family are expressed in cone photoreceptors in a pattern consistent with a role in cone differentiation. In particular, cone photoreceptors uniquely express one

of the receptors (FGFR4) that is highly specific for only one member of the FGF family.

#### **Blood vessel growth during foveal development**

Allende, Madigan, Provis (ANU).

Other studies by our group show that certain members of the Transforming Growth Factor (TGF) family are expressed in the developing retina in a gradient and may have a role in defining the avascular macular region. We are currently testing the hypothesis that TGF $\beta$  and its receptors are highly expressed in this region and inhibit angiogenesis.

#### **Retinal microglia and candidate receptors for HIV-1**

Pham, McCluskey (St Vincents Hospital), Penfold (ANU), van Driel, Madigan.

Microglia are the primary antigen presenting cells in the retina and can harbour viral antigens that may damage neural tissue via the release of neurotoxins. All cells bearing CD4 molecules and co-receptors (members of the chemokine receptor and Fc $\gamma$  receptor families) are potential targets for the human immunodeficiency virus (HIV-1). These studies examined cultured human retinal microglia and frozen sections of human retinas for expression of candidate HIV-1 binding receptors, including CD4, CC chemokine receptor 5 (CCR5) and Fc $\gamma$  receptors. Human retinal microglial cells are found to express detectable levels of CD4, CD16, CD64 and CCR5 in vitro and Fc $\gamma$  receptor I (CD64) in situ. As such, human retinal microglia may be a potential reservoir for HIV-1 infection and be involved in the pathogenesis of HIV retinopathy.

#### **In vivo effects of retinoids on tumour growth in an Rb mouse model**

Madigan, Conway, van Quill (Ocular Oncology, UCSF, San Francisco), O'Brien (Ocular Oncology, UCSF, San Francisco).

These collaborative studies are investigating the efficacy of various retinoids in controlling intraocular tumour growth in a mouse model of Rb. These agents can induce tumour cell differentiation and inhibit tumour growth in some instances. When used in combination with chemotherapy and radiation, some of these agents may reduce the dose of potentially cytotoxic therapies required when treating tumours.

#### **Targeted immunoradiotherapy of ocular melanoma**

Li, Madigan, Conway, Billson, Allen (St George Hospital, UNSW).

Laboratory studies are focused on using select antibodies or proteins to target alpha-radiotherapy more precisely to melanoma cells, so as to treat both primary and metastatic disease. We recently investigated melanoma specific antigen NG2 (using

Mab 9.2.27), which may have potential as a target for radiotherapy treatment. Preliminary studies show that the majority of primary choroidal melanomas and ocular melanoma cell lines (Prof Jager, Leiden, Netherlands) express NG2 immunoreactivity. In vitro studies indicate specific cytotoxicity of alpha-immunoconjugates (213Bi-9.2.27) for NG2-positive melanoma cell lines, suggesting potential use in local therapy either alone or in combination with plaque radiation. An animal model of ocular melanoma is currently being developed to test the effectiveness of targeted alpha-immunoconjugates in controlling tumour growth.

#### **Matrix metalloproteinases in ocular melanoma**

Madigan, Lai, Crouch (SEALS Anatomical Pathology, Prince of Wales Hospital), Conway, Jager (Leiden Medical Centre).

Matrix metalloproteinases (MMPs) are a family of enzymes involved in degradation of extracellular matrix components such as collagen, laminin and proteoglycans. MMPs are involved in normal development, wound healing, new blood vessel growth and tumour invasion and metastases. Our studies in ocular melanoma indicate involvement of MMP-1, -2, -9 and MT1-MMP in tumour angiogenesis and tumour growth. We have also found that ocular melanoma cells express EMMPRIN, an inducer of MMP activity. The juxtaposition of EMMPRIN-positive tumour cells and MMP-2 expressing fibroblasts at tumour edges provides insight into potential mechanisms of ocular melanoma growth and invasion. In vitro studies support these findings, where co-cultures of fibroblasts and melanoma cells display enhanced MMP-2 activity compared to fibroblasts or melanoma cells alone. The expression patterns of MMPs in detached retinas overlying ocular melanomas also suggests an important role for MMPs in retinal gliosis and potentially in neurite outgrowth.

#### **The effect of UV on growth of uveal ocular melanoma**

Lai, Di Girolamo (UNSW), Conway, Jager (Leiden) and Madigan.

Epidemiological studies suggest that exposure to UV radiation has a role in the pathogenesis of ocular melanoma. Preliminary studies have investigated the effects of UV-B radiation on ocular melanoma cell growth, viability and MMP-production. Melanoma cells appear to be very sensitive to UVB radiation and cell death is induced even at very low doses (~5mJ/cm<sup>2</sup>). However, lower doses of UVB do not appear to induce significant effects on MMP production by ocular melanoma cells. In cutaneous melanoma, UVB induces regulation of DNA damage-response genes that may be important for malignant transformation; whether similar changes can occur in normal choroidal melanocytes exposed to low levels of UVB, is currently being investigated.

### **Development of an artificial eye**

Conway, Ben-Nissan (UTS).

The consequence of some ocular diseases, especially tumours, may be the loss of an eye with significant functional and psychological sequelae. Hydroxyapatite is currently used to replace eyes lost due to any cause, however it may be associated with problems including the potential for extrusion, infection, reduced motility and loss of cosmesis over time. The group has developed a novel porous bioceramic with improved biointegration and mechanical properties, which promises better function and cosmesis over existing implants. The results of the physical studies have recently been published; in vivo evaluation is in progress.

### **Retinal Therapeutics Research Group**

This group aims to understand how the permeability of the retinal blood vessels is controlled in order to identify new and improved treatments for swelling of the retina, which is a common cause of loss of vision in conditions such as diabetic retinopathy. The group includes both clinical and laboratory research units.

#### **Group Members**

Mark Gillies, MBBS PhD FRACO, *Unit Director*

#### **Clinical Research Unit:**

Maria Williams, RN BN BA GDip (Acute Care Nurs)  
*Clinical Research Officer*

Haipha Ali, BSc (Applied Vision Sciences, Orthoptics)

Christine Gaston, MBBS, *Clinical Research Officer*

Meidong Zhu, MD PhD, *Senior Research Fellow*

#### **Laboratory Research Unit:**

Martin Windsor, BSc PhD, *Senior Research Fellow*

Li Wen, MBBS MMed, *Research Officer*

Bryony Stracey, *Technical Officer*

Svetlana Cherepanoff, BMedSci MBBS, *Postgraduate Student*

Goff Quin, MBBS, *Postgraduate Student*

Marina Tretiach, BAppSc, *Postgraduate Student*

Jenny Wyndham, BSc DipEd MS MPH, *Postgraduate Student*

#### **Research Activity**

##### **Clinical Research Unit**

The Retinal Therapeutics Research Unit is an internationally certified clinical trial centre that conducts randomised clinical trials and other clinical and basic research in retinal disease. Eight pharmaceutical company sponsored and six investigator initiated clinical trials were undertaken in the Unit in 2005. These study trials involve research into the treatment and mechanisms of age-related macular degeneration,

diabetic retinopathy, central and branch retinal vein occlusion and macular telangiectasia.

##### **Laboratory Research Unit**

Retinal vascular diseases caused by abnormal leakage of fluid through small blood vessel walls (capillaries) are among the commonest causes of blindness – especially in diabetic retinopathy, retinal vein occlusion, ocular inflammation and the wet form of AMD. The Retinal Therapeutics Laboratory Research Unit studies the biomolecular determinants of leakiness in retinal capillaries using laboratory and animal models. The group also conducts preclinical studies into pharmacological and other therapies for the treatment of retinal vascular disease.

##### **Projects in 2005**

###### **Clinical Projects**

###### **A randomised clinical trial of intravitreal triamcinolone for refractory diabetic macular oedema (TDMO study)**

Gillies, Sutter (Zurich University Hospital), Zhu, Ali, Williams, Gaston, Pasadhika (Visiting Fellow).

Macular oedema is the leading cause of vision impairment from diabetic retinopathy. Laser treatment has been proven effective in reducing the risk of visual loss from diabetic macular oedema and is widely employed, but suffers the deficiency that it is inherently destructive. Progressive loss of vision occurs in up to 26% of patients with diabetic macular oedema despite laser treatment. There have been some uncontrolled and anecdotal reports that an intravitreal injection of triamcinolone (IVTA) may result in reduction of diabetic macular oedema and improved visual acuity. This project is a prospective, double-masked, placebo-controlled randomised clinical trial to conduct a two year, single-centre, clinical trial of the efficacy and safety of intravitreal injections of triamcinolone acetonide for diabetic macular oedema that has failed laser treatment. Sixty-nine eyes of 43 patients participated the study. This trial was conducted from March 2002 to April 2005, when patient closeout was completed. The two year results which will be published in the Journal of Ophthalmology, showed that the steroid injections improved vision in most patients who received it and reduced the risk of further loss of vision. Side effects of the treatment, which include possible infection after the injection, cataract and glaucoma, were detected promptly as they occurred and managed adequately without loss of vision. The results of this study will be directly relevant to patients with type I diabetes since it will provide data for use by clinicians that will reduce the risk of blindness from diabetic retinopathy.

**Open label extension of a clinical trial of intravitreal triamcinolone for diabetic macular oedema (TDMX Study)**

Gillies, Zhu, Ali, Williams, Gaston, Pasadhika (Visiting Fellow).

This study is a three year open-label extension of the TDMO study described above. The study is supported by an NHMRC project grant for 2006-2008. The study commenced in April 2005.

**Chorioretinal venous anastomosis for non-ischaemic central retinal vein occlusion**

McAllister (Lions Eye Institute, WA), Gillies, Mitchell (Westmead Hospital), Zhu, Ali, Williams.

Central retinal vein occlusion (CRVO) is a frequent cause of visual loss, especially in the elderly. Obstruction of the central retinal vein may be partial or complete and can produce non-ischemic or ischemic CRVOs. The non-ischemic form initially is more common and vision is reduced due to retinal oedema. Even where the obstruction is eventually overcome, the prognosis for full return of central vision is poor due to macular damage from chronic oedema. A technique of venous anastomosis between the retina and choroid, using high intensity laser as a means of bypassing the obstruction, has been created and that has been able to improve the success rate to 60% in a small trial over five years. The current project is a three year prospective, multicentre, randomised clinical trial to examine whether the use of high powered laser to create retinal vein bypass will benefit patients with a blockage of the central retinal vein. SSI is one of the main centres for the trial. Twenty three patients were enrolled at our site and all of them reached their endpoints. The data analysis is ongoing.

**Intravitreal triamcinolone versus laser for macular oedema secondary to retinal vein occlusion (IVTARVO study)**

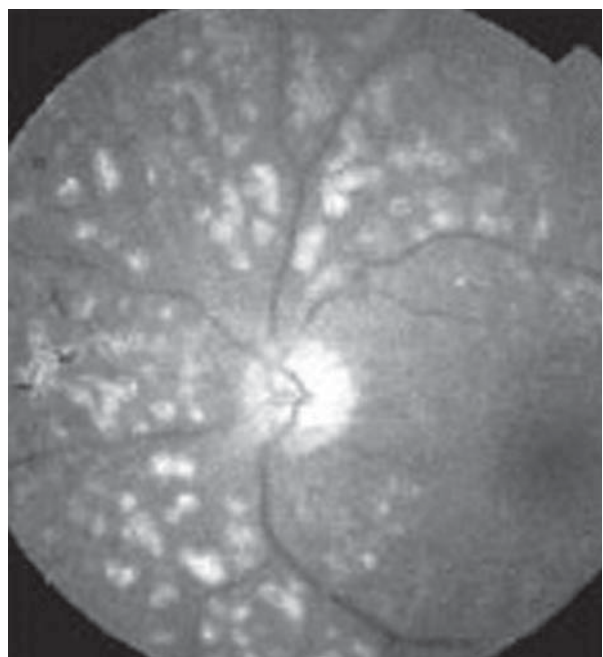
Gillies, Larson (Visiting Fellow), Zhu, Hvarfner, Sutter (Zurich University Hospital), Ali, Williams, Gaston.

An occlusion of a vessel in the retina (retinal vein occlusion) leads to damage of blood vessels, causing them to leak resulting in oedema of the macula. The macular oedema is the main cause of loss of vision in eyes with a retinal vein occlusion. Laser treatment can in some patients improve the visual acuity, but does not work in all cases. We have conducted a randomised clinical trial which has demonstrated that, at least in the short term, injection of a steroid, triamcinolone, into the eye reduces macular oedema and improves vision in 2/3 patients with macular oedema caused by diabetes that had persisted despite laser treatment. In many cases the oedema completely disappeared. This project is to conduct a one year, prospective, multi-centre, double-masked, exploratory, randomised, placebo-controlled trial in patients with branch retinal vein occlusion in

order to assess if intravitreal triamcinolone is superior to laser treatment. Variables related to the progression or resolution of macular oedema will be examined in the study eye of all patients to determine the treatment effects. Five patients reached their end-points in 2005.

**A multicentre randomised clinical trial of laser treatment plus intravitreal triamcinolone for diabetic macular oedema (Thunderbird study)**

Gillies, Zhu, Ali, Williams, Gaston, Pasadhika (Visiting Fellow), McAllister (Uni WA), Smithies (Uni WA), Wong (Uni Melb), McIntosh (Uni Melb), Mitchell (Westmead Hosp), Cho (Westmead Hosp), Arnold (Marsden Eye Centre), Forsyth (Marsden Eye Centre)



Fundus photo of the retina in diabetic retinopathy

This is a Phase II/III, prospective, multi-centre, randomised, double-masked, placebo-controlled clinical trial. This study is supported by an NHMRC Project Grant for 2005-2008. The study is to identify an improved and economical treatment for diabetic macular oedema, one of the commonest causes of blindness both in Australia and the rest of the world.

The specific aims of the study are to test the following hypotheses: that intravitreal triamcinolone followed by laser treatment results in a greater improvement in visual acuity than a placebo (pretend) injection followed by laser treatment of eyes with macular oedema secondary to diabetes; that intravitreal triamcinolone followed by laser treatment results in greater degree of resolution of macular oedema than placebo followed by laser treatment of eyes with macular oedema secondary to diabetes; that intravitreal triamcinolone followed

by laser treatment results in a reduced requirement for further laser treatment to control diabetic macular oedema than placebo followed by laser treatment; and that intravitreal triamcinolone followed by laser has a manageable and acceptable safety profile in eyes with diabetic macular oedema.

The study is currently recruiting patients.

**A natural history study of macular telangiectasia (The MacTel study)**

Gillies, Zhu, Ali, Gaston, Williams.

This natural history study, sponsored by the MacTel Foundation, will characterise the clinical features of type IIa idiopathic juxtafoveal telangiectasia and follow how they change over time. The goal of this study is to develop new treatments for the condition through better understanding of its clinical features. In particular we will identify how loss of vision occurs and investigate whether there is a genetic factor that contributes to the disease. First degree relatives of the participants (primarily siblings; secondarily parents) will also be approached to participate in a family history/genetics sub-study.

Approximately two hundred subjects will be enrolled in the study which will be conducted in up to 25 sites in the United States, Europe, Australia and other selected overseas centres that treat the macular telangiectasia patient population. The Save Sight Institute is one of three study centres in Australia. The participants will be seen at the Sydney Eye Hospital every six months for testing of best-corrected visual acuity, relevant eye examinations and blood tests. The study is for at least five years. Our site enrolled the first patient in the world in October 2005 and currently is still enrolling patients.

**A phase IIIb randomised, double-masked, active controlled, dose-ranging, multi-centre comparative trial, in parallel groups, to compare the safety and efficacy of intravitreal injections of eye001 (pegaptanib sodium, Macugen) given every 6 weeks for 54 weeks, to PDT with visudyne, in patients with exudative age-related macular degeneration (AMD). Protocol EOP1003**

Sponsored by Eyetech Pharmaceuticals  
Gillies, Williams, Zhu, Gaston, Ali, Lake (Visiting Fellow), Pasadhika (Visiting Fellow).

This is a multicentre, international, placebo controlled trial studying the safety and efficacy of an anti-vascular endothelin growth factor aptamer injected into the eye in patients with wet age-related macular degeneration. In 2005 most patients completed an extended third year of treatment. The study was further extended to allow for a fourth and fifth year and two of the remaining eligible patients entered their fourth year.

**A phase II randomised, controlled, double-masked, dose-finding, multi-centre, comparative trial, in parallel groups, to establish the safety and preliminary efficacy of intravitreal injections of EYE001 (anti-VEGF pegylated aptamer) given every 6 weeks for 12-30 weeks to patients with clinically significant diabetic macular oedema (CSME) involving the centre of the macula. Protocol EOP1005**

Sponsored by Eyetech Pharmaceuticals  
Gillies, Williams, Gaston, Zhu, Ali, Lake (Visiting Fellow), Pasadhika (Visiting Fellow).

This is a multicentre, international, placebo controlled trial studying the safety and efficacy of an anti-vascular endothelial growth factor aptamer injected into the eye in patients with diabetic macular oedema. Three patients were enrolled and completed their treatment phase. Post treatment monitoring was completed in early 2005.

**Reduction in occurrence of centre-threatening diabetic macular oedema. Protocol B7A/MC/MBDL**

Sponsored by Eli Lilly Pharmaceuticals  
Gillies, Williams, Zhu, Gaston, Ali, Lake (Visiting Fellow), Pasadhika (Visiting Fellow).

This is a three year multicentre, international placebo controlled trial of an oral protein kinase C beta isoform inhibitor for patients with diabetic macular oedema. The study commenced in 2004 and seven patients have been enrolled into the study. The patient enrolment is still ongoing.

**A Phase III, multicentre, randomised, double-masked, active treatment-controlled study of the efficacy and safety of rhuFab V2 (Ranibizumab) compared with Verteporfin (VisudyneR) photodynamic therapy in subjects with predominantly classic subfoveal neovascular age-related macular degeneration**

Sponsored by Novartis Pharmaceuticals  
Gillies, Williams, Gaston, Zhu, Ali, Lake (Visiting Fellow), Pasadhika (Visiting Fellow).

This two year trial is studying the safety and efficacy of the anti-VEGF compound rhuFab V2 injected intravitreally compared to standard photodynamic therapy with verteporfin in patients with neovascular age-related macular degeneration. The study was activated in June 2004. Enrolment closed in 2004 and the treatment phase remains ongoing.

**A phase 1/2, randomised, masked, single and multiple-dose, sequential dose-escalation study of the safety and efficacy of AG-013958 in subjects with subfoveal choroidal neovascularization associated with age-related macular degeneration. Protocol A4321001**

Sponsored by Pfizer  
Gillies, Williams, Gaston, Zhu, Ali, Lake (Visiting Fellow), Pasadhika (Visiting Fellow).

Commencing in 2005, this study determines the safety and efficacy of multiple sub-tenon injections of A-G 013958, a selective inhibitor of the tyrosine kinase activity of vascular endothelial growth factor 2 (VEGF-R2) in patients with age-related macular degeneration. Four patients were enrolled and the treatment phase is ongoing.

**A phase II randomised, dose ranging, double masked, multi-centre trial, in parallel groups, to determine the safety, efficacy, and pharmacodynamics of intravitreal injections of pegaptanib sodium compared to sham injections for 30 weeks in patients with recent vision loss due to macular oedema secondary to CRVO. Protocol EOP1011**

Sponsored by Eyetech Pharmaceuticals  
Gillies, Williams, Gaston, Zhu, Ali, Lake (Visiting Fellow), Pasadhika (Visiting Fellow).

This 1 year trial of intravitreal injection of pegaptanib sodium compared to sham injection commenced in 2005. The trial assesses the safety and efficacy of the study drug in patients with central retinal vein occlusion. Two patients were enrolled into the treatment phase and the study continues.

**An open label, non-comparative protocol for the use of pegaptanib sodium injection every 6 weeks in patients with exudative age-related macular degeneration (AMD). Protocol EOP1010**

Sponsored by Eyetech Pharmaceuticals  
Gillies, Long, Pasadhika (Visiting Fellow).

This new protocol commenced in 2005 for the compassionate use of the investigational drug pegaptanib sodium "Macugen" for age-related macular degeneration, with patients receiving six weekly intravitreal injections. Large patient numbers are enrolled and this protocol is being administered through Sydney Eye Hospital.

**A six-month phase 3, multicentre, masked, randomised, sham-controlled trial (with six-month open-label extension) to assess the safety and efficacy of 700µg and 350µg dexamethasone Posterior Segment Drug Delivery System (DEX PS DDS Applicator System) in the treatment of patients with macular oedema following central retinal vein occlusion or branch retinal vein occlusion. Protocol 206207-008**

Sponsored by Allergan  
Gillies, Williams, Gaston, Zhu, Ali, Lake (Visiting Fellow), Pasadhika (Visiting Fellow).

This new trial commenced in 2005. Patients with central and branch retinal vein occlusion receive a slow release pellet of dexamethasone, inserted in the eye (or sham procedure) at baseline and are followed for six months. At six months all patients receive an active

dexamethasone pellet and are followed for another six months. Seven patients are enrolled.

## Laboratory Projects

### The effect of paravascular cells on retinal permeability

Tretiach, Gillies.

It is likely that cells situated near the retinal blood vessels can influence permeability. In this project, Müller cells and pericytes have been co-cultured in our laboratory model of retinal vascular permeability. In addition to assessing the effects of these cells on vascular permeability, the effects of various conditions that mimic human disease such as low oxygen levels have also been assessed.

### Autoantibodies in macular degeneration

Cherepanoff, Gillies.

Antibodies directed against retina are known to occur in age-related macular degeneration. This project will determine whether these antibodies have any effect on clinical outcome. A range of patient sera is in the process of being screened and the results will be correlated with the visual outcomes.

### The effect of retinal laser treatment and pharmacological intervention on the permeability of retinal vessels

Quin, Windsor, Tretiach, Gillies.

While laser treatment of retinal swelling is often a very effective treatment, its mode of action is not at all understood. We propose that laser treatment induces the secretion of a protein that stabilises the blood-retinal barrier. Preliminary laboratory experiments in which various retinal cell types are added to the vascular permeability assay suggest that this may indeed be the case. An animal model of early leak in the diabetic rat retina has been characterised to explore the effects of retinal laser treatment more fully. The identification of a barrier-restoring factor may have a therapeutic potential.

### The role of matrix metalloproteinase (MMPs) in the control of retinal vascular permeability

Wyndham, Gillies.

MMPs are a group of enzymes that can digest extracellular materials as well as cell surface receptors. The presence of MMPs was characterised in retinal vascular cells. The results indicate that MMP activity may be involved in the development of retinal swelling in diabetes, and that matrix metalloproteinase inhibitors may represent a new treatment for diabetic retinopathy



### **The role of oxidative damage in diabetic retinopathy**

Van Reyk (UTS), Gillies.

Whilst it is suspected that oxidative stress may contribute to diabetic retinopathy, the identification of those antioxidants that could be developed as a treatment is hampered by the lack of understanding of which of the many potential oxidative pathways in the retina is involved, and precisely what is being damaged. We are using high performance liquid chromatography to detect the target types of oxidative damage to retinal proteins. This will identify the critical pathways involved which will then be targeted by specific antioxidants in animal studies.

### **Connective tissue growth factor and retinal vascular disease**

Wen, Gillies.

CTGF is a recently characterised growth factor which has an activity consistent with the regulation of blood vessel growth. We have found that retinal vascular cells become leakier when treated with CTGF. We have also found that CTGF increases the sensitivity of choroidal vascular cells to the leak-inducing factor VEGF. These findings suggest that CTGF may have a role in the development of vascular eye disease.