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.

Table of Contents

Introduction 1
Table of Contents 1
Corneal Research Group 1
Digital Media Research Group 3
Eye Cancer Research Group 4
Eye Genetics Research Group 5
Electrophysiology and Glaucoma Research Group 6
Inflammatory Eye Disease Research Group 7
Lens Research Group (Cellular and Developmental Biology) 8
Retinal Cell Death and Survival Research Group 10
Retinal Development and Ageing Research Group 11
Retinal Therapeutics Research Group 12
Visual Aging Research Group 18
Visual Neuroscience Research Group 19

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
Gerard Sutton MBBS MD FRANZCO FRACS, Clinical Associate Professor
John Males MBBS BSc FRANZCO, Associate Lecturer
Raj Devasahayam BAppSc, Eye Bank Laboratory Manager
Meidong Zhu MBBS MMed PhD, Eye Bank Senior Hospital Scientist
Mitchell Lawlor MBBS MMed(OphthSci), Postgraduate Student
Athena Roufas MBBS, Research Associate

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 over 400 penetrating corneal grafts to the patients of NSW in 2009. 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 a current average of 9 months. The laboratory staff of the Eye Bank is also investigating methods of corneal storage that extend the life of corneal transplants.

Projects in 2009

Human herpes simplex virus (HSV) and herpes zoster virus
McClelland, Petsoglu

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. The results of the distribution of human herpes viruses (herpes simplex and varicella zoster), including viral loads, in human trigeminal ganglia and corneas, have recently been submitted for publication.

Corneal storage
Devasayaham, Zhu, McClellan, Petsoglu

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.

Utility of corneas from donors with previous intraocular surgery
Zhu, Devasayaham, Georges, Mckeon, McClellan, Petsoglu

The Lions NSW Eye Bank is conducting a study, with the support of Lions NSW-ACT Save Sight Foundation, to determine whether donor eyes with previous intraocular surgery can be included in the donor pool for penetrating keratoplasty (PK) without compromise of patient safety and vision. The study also aims to provide information about the corneas from eyes that had previous ocular surgery.

Human corneal endothelial cell culture
McClelland, Petsoglu, Zhu

In vivo, corneal endothelial cells do not normally replicate. They are essential in the maintenance of corneal clarity and vision. This project aims to define the conditions where human corneal endothelial cells can replicate in vitro. It is hoped that this research will lead to the production of a function cornea utilizing patients' own cells thus preventing the risk of rejection.

Bone marrow derived mesenchymal stem cells in corneal reconstruction
Zhu, Petsoglu, McClellan, Billson

Corneal epithelium and endothelium are critical components of corneal structure. Many corneal diseases result in permanent visual impairment. Although corneal transplantation has a high success rate in treatment of these diseases, donor shortage and corneal grafts rejection remain prominent problems in this area. Limbal stem cells have been considered as a source for continual renewal of corneal epithelium; however, there is no reliable source for corneal endothelium.

Bone marrow mesenchymal stem cells (MSCs) are defined as self-renewable, multipotent progenitor cells with the capacity to differentiate into several distinct mesenchymal lineages. These cells can be isolated from bone marrow, cultured and differentiated in vitro or in vivo. They have generated a great deal of interest because of their potential use in regenerative medicine and tissue engineering. The research group in Save Sight Institute is exploring the possibilities that MSCs isolated from normal rats will be able to differentiate into corneal epithelial and endothelial cells in certain conditions and environments. Their presence will allow damaged corneal epithelial and endothelial cells to be repaired or regenerated. Currently, the group has successfully achieved corneal epithelial cells from bone marrow derived MSCs.

Keratoconus and Wnt Signalling Pathways
Sutton, Roufas, Madigan, McAvoy

Keratoconus (KC), the bilateral progressive, noninflammatory degeneration of the cornea is associated with decreasing visual function. Visual function is lost due to progressive corneal thinning and development of irregular refractive error. The aetiology of KC is unclear but recent studies indicate a role for oxidative damage and keratocyte apoptosis. Keratoconus is the major indication for corneal transplantation in Australia and affects 1 in 2000 in the population. Our studies are investigating Wnt signalling pathways in KC. To
date these studies indicate significant upregulation of SFRP1 in epithelium from KC patients compared to controls. Investigations are currently exploring other Wnt signaling pathway proteins and their role in KC progression.

The Keratoconus research group has patented a diagnostic and therapeutic application for modulation of the Wnt pathway for the treatment of keratoconus. Whilst the research is in its infancy, the group has published their results in the peer-reviewed literature and presented the novel finding at international meetings.

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 develop cutting edge ophthalmic teaching services to provide excellence in education of future eye doctors.

Group Members

John Grigg MBBS FRANZCO FRACS, Unit Head
Anthony Succar, Postgraduate Student

Research Activities

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.

Projects in 2009

Virtual Ophthalmology Clinic
Succar, Grigg

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 progress to 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 practicing on real patients. Its success as a teaching and self-development tool highlights the direction of future digital and distance learning initiatives.

Eye Cancer Research Group

Group Members

Michele Madigan BOptom PhD, Basic Science
R Max Conway PhD FRANZCO, Clinical and translational research
Natalie Walsh-Conway PhD, Research Associate
Yong Li BSc PhD, St George Hospital, UNSW
Kenneth Lai BMedSci, Postgraduate Student
Sarah Rahman BOptom, Honours Student (UNSW)
Jennifer Chow BOptom, Honours Student (UNSW)

Research Activity

We are studying ocular melanoma (affecting choroid, ciliary body, iris and conjunctiva) and retinoblastoma (Rb) (derived from retinal neuroblasts), the most common primary intraocular eye cancers in adults and children respectively. Our studies are investigating some of 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 chemotherapy, and the high incidence of untreatable metastases that are characteristic of ocular melanoma.

Projects in 2009

Effects of histone deacetylase inhibitors (biological response modifiers) on growth and viability of ocular melanomas
Madigan, Conway, Cuneen

These studies are investigating the efficacy of various biologic response modifiers such as histone deacetylase inhibitors and interferons in controlling intraocular, ocular surface and metastatic tumour growth. These agents are very low in toxicity compared to standard chemotherapeutic drugs and can control tumour growth by inducing tumour cell differentiation and inhibiting cell cycling. Importantly, these agents do not seem to affect normal neighbouring cells such as fibroblasts and melanocytes. When used in combination with chemotherapy and radiation, some of these agents can potentially reduce the dose of chemotherapeutic agents required when treating tumours.
Matrix metalloproteinases (MMPs) and MMP-Inducing proteins in ocular melanoma
Madigan, Lai, Crouch (SEALS Anatomical Pathology, Prince of Wales Hospital), Walsh-Conway, Conway

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. Tissue inhibitors of MMPs (TIMPs) can block the activity of MMPs but are also important in cell death and angiogenesis. Our studies in ocular melanoma indicate involvement of MMP-1, -2, -9 and MT1-MMP and TIMP-2 and -3 in tumour angiogenesis and progression. We have also found that ocular melanoma cells express EMMPRIN, an inducer of MMP activity. 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 and TIMPs in detached retinas overlying ocular melanomas also suggest an important role for MMPs in retinal gliosis and in neurite outgrowth during retinal pathology. The techniques developed in these studies are being applied to studies of prostate and ovarian cancer growth and metastases in collaboration with Dr Yong Li (St George Hospital, UNSW) and Prof. Pam Russell (Oncology Research Centre, Prince of Wales Hospital).

Hyaluronan receptors (CD44s) and EMMPRIN in ocular melanoma
Walsh-Conway, Rahman (UNSW), Chow (UNSW), Li (UNSW), Conway, Madigan

CD44 are a group of cell membrane proteins that are involved in cell-cell and cell-matrix adhesion and have been implicated in tumour cell-extracellular matrix (ECM) adhesion and metastatic spread in various malignancies including cutaneous melanoma. CD44s is a receptor for several ECM proteins including laminin and hyaluronan; hyaluronan is critical for tumour invasion and its production is also upregulated by EMMPRIN. Our studies have found expression of CD44s in primary choroid and iris melanomas, including tumour cell membrane and cytoplasmic CD44 immunoreactivity. Moderate to strong immunolabelling (grade 2 or more) could be detected in ~70% of tumours, more commonly in mixed/epithelioid tumours (more aggressive). Tumour vasculature did not appear to be CD44 immunoreactive. We are currently studying interactions between CD44s, EMMPRIN and lactate transporters MCT-1 and MCT-4. Interactions of these proteins appear to be critical in tumour growth and invasion, and targeting some or all of these proteins may be useful therapeutically.

In vivo effects of retinoids and beta-laprapchoine 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 and other biological modifiers including beta-laprapchoine in controlling intraocular tumour growth in a mouse model of Rb. These agents can induce tumour cell differentiation or cell death, and inhibit tumour growth in some instances. When used in combination with chemotherapy and/or radiation, these agents can reduce the dose of cytotoxic therapies required when treating tumours.

The expression and distribution of MUC18 in uveal melanoma
Lai, Conway, Jager (Leiden), Madigan

The immunoglobulin superfamily protein MUC18 is involved in transendothelial migration and signal transduction, and is expressed in malignancies including cutaneous melanoma. Recent in vitro studies showed evidence of increased MUC18 protein in some uveal melanoma cell lines with an increased potential for invasion. We investigated uveal and metastasis-derived melanoma cell lines, normal melanocytes and primary human uveal melanomas for the expression of MUC18 mRNA and protein by RT-PCR, and immunoblotting and immunohistochemistry respectively. Uveal and metastasis-derived melanoma cell lines and primary uveal melanomas expressed variable levels of MUC18 protein. More aggressive primary mixed and epithelioid cell tumours generally expressed more MUC18 than spindle cell tumours, suggesting a role for MUC18 in the growth of more aggressive uveal melanomas. We are currently trying to define the signaling pathways and role of transcription factors including AP-2a in regulation of MUC18 expression in uveal melanoma. Our observations in primary tumours indicate interactions between MUC18-positive melanoma cells and vasculature may be important for the haematogenous spread of tumor cells during metastases. In ocular melanoma, ~40% patients develop liver metastases, with survival of less than 12 months following detection.

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.

Eye Genetics Research Group

Group Members

Robyn Jamieson MBBS FRACP PhD, Unit Head
John Grigg MBBS FRANZCO FRACS
Frank Billson MBBS FRANZCO FRACS FAC FRCPht
Rebecca Storen BSc, Research Assistant
Yongjuan Chen BSc PhD, Postdoctoral Fellow (CMRI)
Linda Weaving BSc PhD, Postdoctoral Fellow (CMRI)
Wan Yi Ng MBBS (Hons), Postgraduate Student (CMRI)
Luke St Heaps BSc (Hons), P/T Postgraduate Student (CHW)
Rebecca Greenlees, Honours Student

Research Activity

Genetic eye disorders contribute to blindness and partial-sightedness for many with visual disability in our community. Our research studies in genetic eye disease focus on 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. 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 treatments to vastly improve the management of these conditions.

In this research programme we are studying patients and families who have 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).

Projects in 2009

The genetic basis of microphthalmia and anophthalmia
Jamieson, Grigg, Storen, Chen, Greenlees, Billson

Microphthalmia (small eye) and anophthalmia (absent eye) cause significant visual disability and the associated features including cataract and glaucoma also contribute to this vision impairment. The underlying genetic causes are unknown in the majority of cases. In a family with two children affected with anophthalmia, we discovered a novel mutation in the SOX2 gene. One of the normal parents was found to be mosaic for the gene change. Hence, SOX2 mosaicism in a parent can lead to anophthalmia recurrence in a family. Our investigation of two mouse models with microphthalmia, indicates a role for the regulation of Wnt signaling in eye development, particularly in retinal and iris development.

High-resolution genomic studies in cataract, glaucoma, anterior segment dysgenesis and retinal dystrophies
Ng, Weaving, St Heaps, Storen, Billson, Grigg, Jamieson

The human genome project has led to rapid advances in analysis of the human genome. We are using high-resolution genomic techniques to identify novel disease genes in patients with eye disorders including cataract, glaucoma, anterior segment and retinal abnormalities. Several novel candidate disease genes have been identified and are undergoing characterization. Cell-based assays and production of animal models are being undertaken for investigation of strong candidates.

Genetics of macular dystrophy
Jamieson, Grigg, Ng

The macula is in the centre at the back of the eye, and is essential for detailed and colour vision. Many elderly patients suffer from macular degeneration. In some familial cases, genes important in maintenance of macular health have been identified. We have ascertained several families with macular disease, where the genetic cause is not known. We are undertaking detailed characterization of the macular features and genetic investigations to identify candidate disease genes.
Electrophysiology and Glaucoma Research Group

Group Members

Alex Klistorner BMed PhD, Unit Co-Head
Stuart Graham PhD MBBS MS FRANZCO FRACS, Unit Co-Head
John Grigg MBBS FRANZCO FRACS
Frank Billson MBBS FRANZCO FRACS FAC FRCOphth
Alessandra Martins MBBS, Postgraduate Student
Hemamalini Srinivasan, Glaucoma Clinical Fellow, Sydney Eye Hospital
Asya Klistorner, BMed Sci, 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 2009

Development of Objective Perimetry – the Multifocal Visual Evoked Potential
Klistorner, Graham, Grigg, Billson

The Save Sight Institute continues to work on development of multifocal visual Evoked Potentials. New binocular system, incorporating dual LCD monitors was designed and built in cooperation with Macquarie University. System will allow eye monitoring, which was not possible previously and therefore expected to improve accuracy of binocular mfVEP

Development of a Blue/Yellow Multifocal Visual Evoked Potential
Martins, Klistorner, Graham, Arvind

Research is also continues into 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. Study was completed in 2009 investigating power of Blue-on-Yellow mfVEP in detecting pre-perimetric glaucoma. Technique demonstrated high sensitivity in cases where subjective perimetry was still normal. Longitudinal (5 years) research is now underway to investigate predictive power of Blue-on-Yellow mfVEP in glaucoma as compare to other structural and functional methods.

Multifocal Visual Evoked Potentials in Optic Neuritis
Grigg, Klistorner, Graham, Garrick, Arvind

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. We conducted study in 2009 looking at demyelination in optic nerve after episode of optic neuritis and possible mechanisms, which may affect remyelination and are currently investigating relationship between inflammation, demyelination and axonal loss using optic neuritis as a model of multiple sclerosis.

Inflammatory Eye Disease Research Group

Group Members

Peter McCluskey MBBS MD FRANZCO FRACS Director SSI & Unit Head
Denis Wakefield MBBS MD DSc FRACP FRCPA Immunologist, UNSW and Sydney Eye Hospital
Nick di Girolamo BSc PhD Research Scientist, UNSW Athena Roufas MBBS Eye Registrar and Research Fellow
Belinda Leong MBBS Eye Registrar and Research Fellow
John Chang MBBS PhD Eye Registrar and Research Fellow
Ed Hughes MBChB Retinal Fellow Sydney Eye Hospital

Research Activity

The Inflammatory Eye Disease Research Group is a new group for the Save Sight Institute which began in 2009
with the appointment of Professor Peter McCluskey to the Save Sight Institute as its new Director.

In 2009, a number of clinical studies were undertaken using patients from the Save Sight Institute and Sydney Eye Hospital campus. At the same time, the research group has been progressing towards its longer term goal of establishing a DNA and patient database of patients with inflammatory eye disease.

Projects in 2009

Conjunctival Triamcinolone for Patients with Non-Necrotising Scleritis
Roufas, McCluskey

This retrospective interventional case series determined the outcomes of 25 sub-conjunctival injections of Triamcinolone in 12 patients who had failed systemic therapy for non-necrotising anterior scleritis. Complete resolution of scleritis occurred in 23 out of the 25 treated eyes. There were no significant complications. Patients were followed up for a mean of nine months following intervention and 40% of treated eyes required repeat injections during the time of followup. This study provides evidence that sub-conjunctival Triamcinolone is an efficacious treatment with a prolonged duration of effect in patients with non-necrotising anterior scleritis who have failed to respond to their initial therapy.

10 Year Outcomes of Graft-Free Molteno Drainage Device Insertion
McCluskey

This study reported the 10 year followup data on 34 patients who had a molteno glaucoma drainage device inserted using a novel surgical technique that does not require an overlying donor scleral graft. At 10 years, the modified surgical technique was shown to be safe with no cases of molteno tube migration or exposure. At 10 year’s followup, 40% of the 34 treated patients, had a functioning successful glaucoma drainage device. This study shows that in long-term followup, the modified, simplified technique is not associated with additional complications, and provides equivalent intraocular pressure control to other surgical techniques. The main advantage of the modified technique is the elimination of the need for donor scleral grafting which eliminates the potential risk of Prion transmission.

Increased Frequency of Retinitis in Syphilitic Uveitis with HIV Co-Infection
Hughes, McCluskey

This study reports the outcomes of 13 consecutive patients with syphilitic uveitis managed by members of the unit. There has been a resurgence of new infections with syphilis in our community over the past five years and this study presents 13 patients, 12 of whom were male and 6 of whom had co-infection with HIV infection. Men who have sex with men were the major risk group in this patient population. Patients developed characteristic retinitis and severe uveitis which responded rapidly and well to intensive antibiotic and anti-inflammatory therapy. This study reinforces the importance of considering syphilis in the differential diagnosis of many different ocular presentations.

Tuberculous Uveitis in the Multicultural Australian Population
Leong, McCluskey

This study, which is still ongoing, seeks to determine the diagnostic criteria and management of patients with uveitis associated with TB. TB-related eye disease has become increasingly diagnosed in Australia despite the low prevalence of TB in our community. The majority of patients developing TB related eye disease in Australia have migrated from endemic high-prevalence TB areas. Thus far, we have data on 44 patients who have been diagnosed with TB-related uveitis and inflammatory eye disease.

We are continuing to accumulate patients and hope to have sufficient data to report this study in late 2010. At this time, it appears that TB-related uveitis and inflammatory eye disease is under-diagnosed in the Australian population. It appears that good visual outcomes and decreased rate of recurrence can be achieved with prompt diagnosis and treatment.

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
Yuki Sugiyama BSc PhD, Postdoctoral Fellow
Richard Stump BSc PhD, Laboratory Manager
Jessica Boros BSc, Research Assistant
Anke Nguyen BSc, Research Assistant
Colin Chong MBBS, Postgraduate Student
Kevin Wang BSc, Postgraduate Student
Hailey Shin BSc, Postgraduate Student
Ana Tanedo, Honours Student
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. Based on this we proposed that an anterior-posterior gradient of FGF determines lens polarity and growth patterns in vivo. There is now compelling evidence to support this model and a major thrust of our research activity is aimed at elucidating FGF-induced signaling pathways and details of their regulation.

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 (TGFβ) 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 TGFβ 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.
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, Wang

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 signalling 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**
Sugiyama, Stump, McAvoy, Lovicu

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 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.

**Wnt signalling in lens development**
Sugiyama, Stump, Nguyen, 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, key Wnt/Fz signalling molecules, including those involved in Wnt/ß-catenin and Planar Cell Polarity (PCP) pathways, are expressed in the lens. A mouse mutant that is deficient in a co-factor required for Wnt/ß-catenin signalling does not develop a complete lens epithelium. In addition, a transgenic mouse that overexpresses an inhibitor that blocks all Wnt signalling pathways, specifically in the lens shows disturbed alignment/orientation of fibres. Downregulation/inhibition of PCP signalling components in lenses of these mice is consistent with this pathway having a key role in coordinating the three-dimensional cellular architecture of lens. Direct evidence that a PCP mechanism operates in lens comes from the observation that most lens cells have primary cilia that are polarized towards the lens anterior pole. Ongoing research aims to determine if these primary cilia are a component of a 'Global Positioning System' that directs the polar migration/orientation of lens fibres. Taken together, these results support our hypothesis that several Wnt signalling pathways play key roles in the differentiation/maintenance of both forms of lens cells.

**Retinal Cell Death and Survival Research Group**

This group is now relocated at the University of Sydney, with a 45% appointment at SSI. It has been associated with the Save Sight Institute since 2002.
One major strand of our work concerns the visual system, particularly the retina; a second major strand concerns the stability of the brain, particularly in relation to age-related dementia.

**Group Members**

Jonathan Stone BSc PhD FAA, Unit Head  
Allison Cameron PhD, Research Student  
Sivaraman Purushothuman BSc (Hons), Research Assistant  
Sally Stowe PhD, Research Assistant  
Siliva Bisti PhD (University of L’Aquila), Research Associate

**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 2009**

Current joint projects include the use of dietary antioxidants, near-infrared radiation, and management of oxygen and light levels to optimize photoreceptor stability and slow the progress of degenerative processes. In parallel series of experiments the work is defining the regional and cellular responses of the retina to these forms of environmental manipulation, and is using microarray technologies to identify the signaling pathways involved.

---

**Retinal Development and Ageing Research Group**

**Group Members**

Michele Madigan BOptom PhD, Unit Head  
Jan Provis BSc PhD, SSI Associate (ANU)  
Diana van Driel BSc (Hons), Senior Research Assistant  
Alexandra Allende MBBS, Postgraduate Student  
Luis Munoz-Erazo BSc, Postgraduate Student (USyd Pathology, SSI)

**Research Activity**

The human ‘macula’ is a specialized area of the primate retina that enables us to see acute 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. 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.

**Projects in 2009**

**Photoreceptor degeneration in normal ageing and age-related macular degeneration**  
Madigan, Provis (ANU)

Loss of vision due to AMD is devastating for individuals and families, with very high economic costs to the community. Several lines of evidence indicate photoreceptor dysfunction in early AMD, even when visual acuity is stable, including reduced contrast sensitivity, slowed recovery after photostress, delayed dark adaptation, and reduced amplitude and delayed latency in the foveal electroretinogram (ERG). Our preliminary work and recent functional studies suggest that visual dysfunction in AMD can occur beyond the central retina, where cone photoreceptors primarily function to give colour vision & fine detail vision. This study investigates photoreceptor survival and degeneration across the human retina in normal ageing & AMD, compared with young normal retinas. Specifically,
the early changes in photoreceptors with normal ageing and in AMD are being studied using markers of photoreceptor “wiring” (synapse components), both in central and peripheral regions of the retina. We also address the question of whether degeneration related to normal ageing of photoreceptors is associated with low-level, chronic inflammation in the outer retina. Polymorphisms for genes important for innate immunity (such as Complement Factor H) are also being studied using DNA sequencing. These studies are important for understanding whether low-grade inflammation, combined with these underlying gene alterations, are important for the pathogenesis of AMD.

The Effect of West Nile Virus Infection on Human Retinal Pigmented Epithelium Extra-cellular Matrix Production
Munoz-Erazo, Madigan, Gillies, Provis (ANU), King (Pathology, USyd)

AMD is a leading cause of irreversible blindness in the elderly. The pathogenesis of AMD remains elusive. Genetic polymorphisms in the alternative complement pathway (activated by external pathogens) have recently been identified, associated with a strong susceptibility to developing AMD. Numerous studies also indicate a major role for inflammation in the AMD. One hypothesis suggested by these observations is that for individuals with complement-related genetic polymorphisms, exposure to pathogens such as Chlamydia or viruses, may lead to a chronic dysregulated immune response, associated with impaired outer BRB function, leukocyte and blood vessel invasion, and altered extracellular matrix (ECM) production. We are investigating whether a viral pathogen (West Nile Virus - WNV) can affect RPE production of ECM proteins such as fibronectin, collagen I and IV, and vitronectin. These ECM proteins have been associated with cell proliferation, upregulation of angiogenic factors, and several AMD-related diseases such as proliferative retinopathy. Collagen IV and vitronectin are predominant ECM proteins in basal lamina deposits and drusen associated with retinal ageing. Matrix bound VEGF-A will also be measured, as this is a well known angiogenic factor.

Blood vessel growth during foveal development
Allende, Madigan, Provis (ANU)

Earlier studies by our group showed 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 have investigated the hypothesis that TGFβ and its receptors are highly expressed in the developing foveal region and inhibit angiogenesis, either by inhibiting proliferation and/or migration of retinal endothelial cells and macroglia.

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 Head

Clinical Research Unit:
Haifa Ali, BSc (Applied Vision Sciences, Orthoptics), Clinical Research Orthoptist
Daniel Barthelmes, MD, Research Fellow
Dr Samantha Fraser-Bell, MBBS BSc(Med) MHA MPH FRANZCO, Retinal Specialist
Christine Gaston, MBBS, Clinical Research Officer
Briony Giaustinibury, MHSM, FRB! Project Manager
Ann Gould, BSc, Clinical Research Officer
Amparo Herrera-Bond, DipAppSc (Orthop) BBus, Clinical Research Orthoptist
Grace Hunt, MBBS, Photographer
Dr Alex Hunyor, MBBS(Hons) FRANZCO, Retinal Surgeon
Liudmila Kolmogorova, BMedSc, Research Assistant
Dr Srikanth Narayana, MBBS MS (Ophtho) FVR (RGUHS), Visiting Vitreoretinal Fellow
Dr Rajesh Rajagopalan, MBBS FRCS Ed MS DNB DO, Visiting Vitreoretinal Fellow
Maria Williams, RN BN BA GDip (Acute Care Nurs) Clinical Research Officer
Meidong Zhu, MD PhD, Senior Research Fellow

Laboratory Research Unit:
Daniel Barthelmes, MD, Research Fellow
Svetlana Cherepanoff, BMedSci MBBS, Postgraduate Student
Sook Hyun Chung, BMedSc (Hons), Research Assistant
Narelle Jay, BBiotech (Hons) Postgraduate student
Liudmila Kolmogorova, BMedSc, Research Assistant
Alice Len, PhD, Research Fellow
An Nguyen, BSc (Hons), Research Assistant
Weiyoung Shen, MD PhD, Senior Research Fellow
Ling Zhu, PhD, Postdoctoral Research Fellow

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. Both pharmaceutical company sponsored and investigator initiated clinical
trials were undertaken in the unit throughout the year. One large sponsored natural history study was also ongoing this year. Current trials undertaken 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, the wet form of AMD and type 2 of perimacular telangiectasia. 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 diseases.

**Projects in 2009**

**Clinical Projects**

**A natural history study of macular telangiectasia Type 2 (The MacTel study)**

Gillies, Zhu, Ali, Kolmogorova, Gaston, Williams, Hunt, Herrera-Bond, Gould

This natural history study aims to characterise the clinical features of macular telangiectasia type 2 (MacTel Type 2), to follow the changes over time and to develop new treatments through better understanding of the clinical features. In particular we will identify how loss of vision occurs and investigate whether there is a contributing genetic factor. First degree relatives of participants (primarily siblings; secondarily parents) take part in a family history/genetics sub-study.

**Total 452 subjects were enrolled by the end of 2009 in up to 25 sites in the United States, Europe, Australia and other selected overseas centres. The Save Sight Institute is one of three study centres in Australia. Initially for five years from October 2005, the study has been extended for another 5 years. Our site had enrolled 44 participants by the end of 2009, making us second of 25 sites around the world. In addition, by the end of 2009, we screened 94 family members and accrued 21 control subjects of a total of 316 family members and 79 controls in all 25 sites for the Genetics study. This puts us at number one position in the world for the fourth consecutive year. Our site, which appears to be the first to start screening family members, found more than one family member with the condition in 7 separate families including one family with monozygotic twins. None of these affected family members realised they had the condition since their vision was normal, raising the suggestion that there may be many more family members of affected patients with disease that is not affecting their vision. It also suggests that patients with the condition who have disturbance of vision (ie currently all the participants in the Natural History Study) have an advanced form of this disease. A paper of the research results from the family member study has been published in Ophthalmology 2009. Patient enrolment and family member screening is continuing.**

**The efficacy and Safety of Treatment with Intravitreal Ranibizumab in Patients with Branch Retinal Vein Occlusion ‘L-BRVO’**

Gillies, Gaston, Williams, Gould, Zhu, Ali, Narayana, Rajagopalan

Three Australian sites commenced this study in 2008 which involves patients with Branch Retinal Vein Occlusion being randomized (1:1) to treatment with either intravitreal ranibizumab or Sham (pretend injection) over 12 months. Patients are currently being enrolled. It is envisaged the study will be completed by the end of 2010 or early 2011.

**Clinical trial of Ranibizumab for Diabetic Macular Oedema resistant to Intravitreal Triamcinolone “TAREDS” Study**

Gillies, Gaston, Williams, Herrera-Bond Zhu, Ali, Gould, Narayana, Rajagopalan

Diabetic macular oedema (DMO) is thought to result from a series of biochemical and cellular changes, causing progressive leakage and exudation. Focal and grid photocoagulation remain the standard care for diabetic maculopathy. Availability of new agents raises the possibility of improvements in outcomes; however these benefits must be demonstrated in clinical trials. Vascular endothelial growth factor (VEGF) levels have been found to be elevated in the aqueous and vitreous humour of patients with DMO. We have recently demonstrated reduction in levels of VEGF in a rat model.
of diabetic retinopathy post treatment with IVTA. We believe, however, that specific VEGF inhibitors such as ranibizumab may have a stronger effect on VEGF compared with steroids. This study aims to demonstrate the efficacy and safety of an intravitreal injection of ranibizumab for patients with triamcinolone resistant DMO. Five patients / six eyes have been enrolled. Current results show that four of five patients (five / six eyes) had improved or stable vision with reduction of macular thickness. Patient enrolment is continuing.

Open label extension of a clinical trial of intravitreal triamcinolone for diabetic macular oedema (TDMX Study)
Gillies, Zhu, Ali, Williams, Gaston, Hunt, Simpson (USyd)

Macular oedema is the leading cause of vision impairment from diabetic retinopathy. Progressive loss of vision occurs in up to 26% of patients with diabetic macular oedema despite conventional laser treatment. In 2002, our group commenced a prospective, double-masked, placebo-controlled randomised clinical trial that investigated the efficacy and safety of intravitreal injections of triamcinolone acetonide (IVTA) for diabetic macular oedema that had failed laser treatment (TDMO Study). In the TDMO study sixty-nine eyes of 43 patients participated. The two year results, published in Ophthalmology, showed that the steroid injections improved vision in most patients who received it and reduced the risk of further loss of vision. With NHMRC project funding support 2006-2008, thirty-three patients (57 eyes, including 29 previously IVTA-treated eyes and 28 previously placebo-treated eyes) from the original TDMO study were enrolled in this study. This trial began progressively from the 24 month visit of the TDMO study for each patient. A three year TDMX study for each participant. A three year TDMX follow up has now been completed, in which all the eyes of study participants were treated with medication (intravitreal triamcinolone) as required, as well as standard laser treatment where appropriate. The 5-year results have been published in Ophthalmology 2009, November. This study suggests that the beneficial effect of intravitreal triamcinolone in eyes with DMO persists for up to 5 years in most eyes without a large increase in treatment-related adverse events. We believe that treatment with eye injections of a steroid triamcinolone will be considered in carefully selected cases of impaired vision from advanced diabetic macula oedema which has not responded to usual treatment with laser. This study has now concluded.

A multicentre randomised clinical trial of laser treatment plus intravitreal triamcinolone for diabetic macular oedema (Thunderbird study)
Gillies, Zhu, Ali, Williams, Gaston, Hunt, Kolmogorova, Gould, Simpson (USyd) McAllister, Smithies (Uni WA), Wong, McIntosh, Ewing (Uni Melb), Arnold, Forsyth (Marsden Eye Specialists)

This is a Phase II / III, prospective, multi-centre, randomised, double-masked, placebo-controlled clinical trial. The study aimed to identify how treatment with intravitreal triamcinolone, pioneered by the Save Sight Institute, is best combined with conventional laser photocoagulation for the treatment of diabetic macular oedema (DMO), one of the commonest causes of blindness in both Australia and the rest of the world.

The specific aim of the study was 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 a 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.

Total 84 eyes of 54 participants were entered into the study in the four participating centres - Save Sight Institute (lead centre); Centre for Eye Research Australia, Melbourne; Marsden Eye Specialists, Parramatta, Sydney; Lions Eye Institute, Perth. Of these, 42 eyes were randomly assigned to receive IVTA plus laser and 42 to laser treatment alone. Thirteen eyes withdrew. The 6-month study results were published in IOVS 2009 and 2-years results have been submitted to Ophthalmology for publication. The study results suggest that treatment with IVTA plus laser resulted in a doubling of improvement in vision by ≥10 letters compared with laser only over 2 years. This suggests that IVTA may be a useful adjunct to laser treatment for management of eyes with DMO. This study has now concluded, however some results are still in analysis.
**Fight Retinal Blindness!**

Gillies, Glastonbury, Kolmogorova, Barthelmes (USyd), Wong (Uni Melb), McAllister (Uni WA)

The Fight Retinal Blindness! Project is a collaborative initiative between the Save Sight Institute, Sydney; Centre for Eye Research Australia, Melbourne; and Lions Eye Institute, Perth. The overall goal is to develop strategies to reduce the incidence of retinal blindness in the Australian community, with the early aims being:

1. To monitor, track and evaluate new treatment strategies for AMD to ensure quality patient outcomes by establishing practice-level patient quality management audit; monitoring patient quality of life outcomes; facilitating national performance benchmarking; and developing evidence based clinical management guidelines; and to

2. Undertake linkage of data across population health databases to inform assessment of morbidity and mortality associated with the new treatments for macular disease

The key benefits of the FRB! Project to the community are the immediate improved management of patients with AMD — clinicians will be able to deliver the appropriate treatment, at the appropriate frequency and for the appropriate length of time. Development of research infrastructure will facilitate other significant research into outcomes of treatment of other retinal conditions such as diabetic retinopathy and retinal detachment, and will provide the framework for a similar system to be implemented for a range of ophthalmic conditions.

The three core sites have been operational since late 2008. Data collection is under way in another three practices, one in Sydney and two in Melbourne. The data management system is being redeveloped as an SQL database with a web interface, which will provide significant benefits in the medium to longer term. It will enable local and international sites to easily join the project, facilitate easier upgrades and secure, streamlined data transfer. Additional practices will commence using the new software after onsite functional testing has been completed in mid-June 2010. Performance benchmarking will be facilitated enabling participating centres to easily and anonymously compare their clinical outcomes with those of other centres.

A demonstration data linkage project, with data on approximately 3,000 patients, is under way in WA where a State/Federal level agreement facilitates linkage of population health data sets. The incidence of systemic adverse events in patients who have had an intravitreal injection will be compared with those who have not, to determine if the incidence is greater in that group. Results are likely to be available in late 2010.

**Pharmaceutical Sponsored Trials**

**Diabetic Retinopathy and Diabetic Macular Oedema**

A randomized, double-masked, multicenter, laser-controlled Phase III study assessing the efficacy and safety of ranibizumab (intra-vitreal injections) as adjunctive and monotherapy in patients with visual impairment due to diabetic macular oedema (RFB002D2301 ‘RESTORE’)

AND

A phase IIIb, open-label, multi-center 12 month study to evaluate the safety, tolerability and efficacy of ranibizumab (0.3 mg) in patients with subfoveal choroidal neovascularization secondary to age-related macular degeneration ‘RESTORE’

Sponsored by Novartis

Gillies, Williams, Gaston, Gould, Herrera-Bond, Zhu, Ali, Narayana, Rajagopalan

The initial trial commenced in March 2007 and 6 patients were enrolled. All final study visits were completed in 2009. Three patients continued into the open open-label extension.

A 3-Year, Phase 3, Multicenter, Masked, Randomised, Sham-Controlled Trial 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 Diabetic Macular Oedema. (POSURDEX DME)

Sponsored by Allergan

Gillies, Williams, Gaston, Gould, Herrera-Bond, Zhu, Ali, Narayana, Rajagopalan

This trial compares a slow release pellet of dexamethasone to a sham (pretend) injection which is injected into the vitreous (jelly) of the eye to study its safety and effectiveness in treating macular oedema associated with diabetes. This trial is ongoing, however patients are no longer being recruited.

**Age Related Macular Degeneration (AMD)**

The Safety and Efficacy of AL-8309B Ophthalmic Solution for the Treatment of Geographic Atrophy (GA) Secondary to Age-Related Macular Degeneration (AMD)

Short title: Geographic Atrophy Treatment Evaluation (the GATE study)

Sponsored by Alcon

Gillies, Williams, Gaston, Gould, Herrera-Bond, Zhu, Ali, Narayana, Rajagopalan

A 3 year clinical trial in dry AMD comparing AL-8309B eye drops to placebo eye drops. This trial is ongoing, however patients are no longer being recruited.
A randomized, double masked, active controlled, phase 3 study of the efficacy, safety, and tolerability of repeated doses of intravitreal VEGF Trap-Eye in subjects with neovascular age-related macular degeneration (AMD) (the VIEW 2 study)
Sponsored by Bayer
Gillies, Williams, Gaston, Gould, Herrera-Bond, Zhu, Ali, Narayana, Rajagopalan, Kolmogorova

A 2 year clinical trial in wet AMD comparing VEGF-Trap eye injections and Lucentis injections. This trial is ongoing, however patients are no longer being recruited.

A Multicenter, Masked, Randomized, sham-controlled, Paired-eye Comparison, 12-Month (Plus 12 Month Extension) Study to Evaluate the Safety and Effects on Retinal Structure and Visual Function of Brimonidine Tartrate Posterter Segment Drug Delivery System (Brimonidine Tartrate PS DDS) Applicator System in Patients with Geographic Atrophy from Age-related Macular Degeneration
Sponsored by Allergan
Gillies, Williams, Gaston, Gould, Herrera-Bond, Zhu, Ali, Narayana, Rajagopalan, Kolmogorova

A 2 year clinical trial comparing a slow release pellet of brimonidine tartrate to sham (pretend) injection into the vitreous (jelly) of the eye to study its safety and effectiveness in the treatment of dry macular degeneration. This trial is ongoing, however patients are no longer being recruited.

A 2 year, Multicenter, Randomized, Controlled, Masked, Dose-Finding Trial to Assess the Safety and Efficacy of Multiple Intravitreal Injections of AGN 211745 in Patients with Subfoveal Choroidal Neovascularization Secondary to Age-Related Macular Degeneration. Protocol 211745-001 SIRIUS
Sponsored by Allergan
Gillies, Williams, Zhu, Ali, Gaston, Siow, Hunt, Hughes

This trial commenced in 2007. Patients with subfoveal choroidal neovascularization (CNV) associated with Age-Related Macular Degeneration (ARMD) receive one of four study treatments in a 1:1:1:1 ratio: AGN211745 100µg : AGN211745 300µg : AGN211745 1000µg : Lucentis™ 500µg and will be followed up for 24 months. AGN211745 is a small-interfering RNA (SIRNA) designed to target a specific site in the VEGFR-1 mRNA, therefore, blocking the production of the VEGFR-1 protein and possibly inhibiting the neovascularisation and leakage associated with ARMD. Seven patients were enrolled. The trial was closed in May 2009 by Sponsor.

Central Retinal Vein Occlusion
A randomized, double-masked, sham-controlled phase 3 study of the efficacy, safety, and tolerability of repeated intravitreal administration of VEGF Trap-Eye in subjects with macular edema secondary to central retinal vein occlusion (CRVO) (the GALILEO study)
Sponsored by Bayer
Gillies, Williams, Gaston, Gould, Herrera-Bond, Zhu, Ali, Narayana, Rajagopalan, Kolmogorova

A 2 year clinical trial in Central Retinal Vein Occlusion comparing VEGF-Trap Eye to sham (pretend) injection. Patients are currently being enrolled.

Laboratory Projects

**In vitro modeling of the Blood-Retinal barrier**
Shen, Li, Chung, Gillies

The aim is to establish a reliable in vitro bioassay to model the BRB, and to test the impacts of growth factors such as transforming growth factor beta 1 (TGF-β1) on BRB breakdown. Retinal microvascular endothelial cells are isolated from bovine eyes. In addition, an immortalized brain microvascular endothelial cell line is also used in this study. The effects of TGF-β1 on BRB function are investigated by measurement of the BRB function and analysis of expression of tight junction proteins and their phosphorylations using monolayer of vascular endothelial cells in transwells.

**The role of glial dysfunction in diabetic retinopathy**
Shen, Chung and Gillies

Diabetic retinopathy (DR) is a sight threatening, chronic ocular disorder that develops with time in nearly all people with diabetes. It is linked to hyperglycemia-induced biochemical abnormalities that initiate gradual and progressive alterations in the retinal microvasculature, neurons and glial cells. To date, vascular abnormalities have been the primary target for treatment. However, little attention has been paid to the specific role of glial dysfunction in the pathogenesis of DR. Retinal glia is thought to play an important role in the maintenance of normal retinal function. Reactive changes in Müller glia, such as an up-regulation of glial fibrillary acidic protein (GFAP), occur early in the course of the disease and precede the onset of overt changes in the retinal vasculature and neurons. The causal role of glial dysfunction in DR currently remains unknown.

It is our hypothesis that in individuals with diabetes, prolonged glial dysfunction contributes significantly to the pathogenesis of DR by inducing secondary alterations in the retinal vasculature and neurons that might not otherwise have occurred. This hypothesis will be tested in a novel animal model of retinal glia dysfunction that we have recently developed after intraocular injections of a glial-specific toxin, DL-alpha-
Generation of an inducible transgenic model for Müller cell-specific genetic targeting
Shen, Früttiger, Chung, Nguyen, Zhu and Gillies

Müller glial dysfunction is found in a number of retinal vascular diseases but the link between Müller dysfunction and blood–retinal barrier (BRB) breakdown remains poorly understood. This project aims to develop an inducible transgenic model for Müller cell-specific genetic targeting for future investigations of the role of Müller cells in retinal vascular diseases. We have generated a DNA construct containing a Müller cell-specific promoter driving a tamoxifen inducible form of Cre recombinase (CreERT²). The cell-specific promoter contained a 2kb fragment of regulatory region of the retinaldehyde binding protein 1 gene (Rlbp1). Transgenic mice were generated by pronuclear injection of the DNA construct. The generated Rlbp1-CreERT² transgenic mice were crossed into a Rosa-LacZ reporter background and offspring were screened for LacZ expression in the retina after tamoxifen induction. Two transgenic lines were generated and both displayed moderate and consistent LacZ expression after tamoxifen induction in adult animals. The LacZ expression was specifically localised to Müller cells in the retina. The number of LacZ expressing Müller cells increased after multiple tamoxifen administrations compared with one dose induction. LacZ expression was not observed in the retinal pigment epithelium and other types of retinal cells. Crossing Rlbp1-CreERT² transgenic mice carrying a toxic gene led to selective, patchy ablation of Müller glial activation, retinal neuronal damage, BRB breakdown and vascular telangiectasis. In this inducible transgenic model, retinal neuronal injury can be observed as early as 7 days after Muller glial disruption but severe vascular leakage and deep retinal neovascularization occurred much later, which were commonly observed after 2 months of Muller glial disruption. This inducible transgenic model presents dynamic changes in retinal neuronal injury, the BRB breakdown and retinal neovascularization and will be a useful tool for testing compounds in neuroprotection and anti-angiogenesis therapies.

Erythropoietin treatment stabilizes retinal vasculature and improves vascular lesions in RCS rats
Shen, Chung and Gillies

Royal College of Surgeons (RCS) rats develop progressive retinal neuronal damage and vascular abnormalities. The aim of this study was to investigate if erythropoietin (EPO) is effective in protecting retinal vasculature from damage in RCS rats. Fundus fluorescein angiography (FFA) was employed to monitor dynamic retinal changes in RCS rats from 6 to 25 weeks of age. Confocal microscopy was performed with frozen sections using antibodies for changes in the retinal glia [glial fibrillary acidic protein (GFAP), glutamine synthetase (GS), vimentin and OX-42], retinal vasculature (collagen IV) and expression of vascular endothelial growth factor (VEGF) and pigment epithelium derived growth factor (PEDF). To study the effect of EPO treatment on retinal vascular rescue, EPO was injected intraperitoneally in RCS rats at 14 weeks of age with a dose of 5000IU/kg, twice a week for 4 consecutive weeks. Changes in the retinal vasculature, glia and recruitment of CD34+ endothelial progenitor cells into the retina were examined by FFA and confocal microscopy using frozen sections and flatmounted retinas. RCS rats developed progressive vascular lesions from 3 months of age, which were predominantly confined to regions surrounding the optic disc. Changes in the retinal vasculature were accompanied by progressive disruption of the retinal glia (astrocytes, Müller cells and OX-42+ microglial cells), increased expression of VEGF and decreased expression of PEDF. Immunostaining for collagen IV showed subretinal vascular abnormalities. Quantitative image analysis demonstrated that EPO treatment significantly reduced the area of vascular lesions and stabilized both superficial and deep retinal vascular plexuses. Immunohistochemical studies revealed that EPO reduced retinal glial swelling and increased the number of OX-42+ and CD34+ cells in the inner retina but most CD34+ cells failed to incorporate into the retinal vasculature. Our results indicate that EPO may have valuable therapeutic potentials for retinal vascular rescue via modulating retinal glia and bone marrow derived progenitor cells in pathological conditions.

Role of bone marrow derived progenitor cells in diabetic retinopathy
Barthelmes, Shen and Gillies

Diabetic retinopathy (DR) is a sight threatening, chronic ocular disorder that develops with time in nearly all people with diabetes. It is linked to hyperglycemia-induced biochemical abnormalities which initiate gradual and progressive alterations in the retinal microvasculature at early stage of the disease. Vascular endothelial dysfunction is an early feature in the pathogenesis of DR. With continued progression of diabetes, failure to repair the injured retinal microvasculature results in retinal ischaemia and neovascularisation. Functioning as adult stem cells, bone marrow progenitor cells are capable of differentiating into a variety of cell types including vascular endothelial cells and have physiological roles in the maintenance and rescue of the existing retinal capillary beds. This project aims to determine the involvement of bone marrow progenitor cells in DR and to investigate the feasibility of using bone marrow progenitor cells to repair retinal vascular injury in early stage of DR.
Identifying the pathogenesis of idiopathic macular telangiectasia: A proteomic approach.
Len, Gillies

Idiopathic macular telangiectasia is an uncommon but vision threatening disease, where little is known about the cause and what biomolecular mechanisms are involved. Since the vitreous apposes the retina and acts like a biomolecular ‘sink’ to this component of the eye, we believe that analyzing the vitreous using proteomic techniques may give us insight into what potential disease mechanisms or biomarkers may be involved. The identification of biomarkers will not only further our understanding of the disease but may also provide putative targets for therapeutic intervention.

Proteomics was performed on macular and vitreous samples collected from a MacTel patient (assigned ID 70-99) and results were compared with those obtained from patients with diabetes but without diabetic retinopathy. From the macular samples, a total of 694 proteins were identified by proteomic analysis and 155 proteins were differentially expressed, of which 81 proteins were increased and 74 proteins decreased in 70-99. By analysing the differentially expressed proteins in 70-99, it was revealed that the levels of proteins involved in oxidation phosphorylation were increased globally and those involved in the glycolytic pathway were decreased. From the vitreous samples, collectively a total of 162 proteins were identified, of which 27 proteins were increased and 31 proteins decreased in the MacTel sample compared to control. Several glial cell markers including GFAP and vimentin and a number of glycolytic proteins were increased in the vitreous of 70-99. Results of this study indicate that metabolic uncoupling between Muller cells and photoreceptors may play an important role of the pathogenesis of MacTel.

Wnt and TGF-β signaling in diabetic retinopathy
Zhu, Shen, Chung and Gillies

Diabetic retinopathy (DR) is amongst the leading causes of blindness in the Developed World. Being already the dominant cause in working age people, the incidence of DR is expected to climb dramatically as diabetes mellitus increases globally due to increasing life expectancy and obesity.

Our data showed that 18 weeks after diabetes the two major TGF-β ligands, TGF-β1 and TGF-β3, were significantly up-regulated at the mRNA level in diabetic rats compared with non-diabetic controls. The phosphorylated-Smad2, one of the essential signalling factors for the TGF-β pathway, was significantly increased at protein level. In addition, key factors of the canonical Wnt signalling pathway, including β-catenin (the major Wnt effector protein) and Wnt5a and Wnt3 (essential Wnt ligands) were up-regulated in diabetic retinas when analyzed by RT-PCR and Western blotting.

Taken together, the data from our study indicates that both the TGF-β and canonical Wnt signalling pathways appear to be activated in DR.

Future investigation will elucidate the roles of the TGF-β and Canonical Wnt signalling pathways in the pathogenesis of DR, especially their synergistic effect on the BRB breakdown in early stage of DR. This is of considerable scientific and clinical significance, as the data will allow us to improve current understanding on the molecular pathogenesis of DR, which is currently very limited, as well as to discover new clinical targets in order to treat this disease better in people with diabetes in the future.

Visual Aging Research Group

Group Members
Roger Truscott BSc PhD, Unit Head, NHMRC Senior Research Fellow
Michael Friedrich, BSc PhD, Postdoctoral Fellow
Anastasia Korlimbins Phd, Postdoctoral Fellow
Jane Deele, Postgraduate Student (Uni Wollongong)
Brian Lyons, Postgraduate Student
Jessica Nealon, Postgraduate Student (Uni Wollongong)
Jim Shi-Ping, Postgraduate Student (Uni Wollongong)
Michelle Hooi, Postgraduate Student

Research Activity

Research is focused on two common ocular 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.

Our data indicate 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 lifetime. 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 2009**

**UV filter Binding to Lens Proteins with Age**
Lyons, Korlimbinis, Truscott

In 2006 we developed a novel method for measuring the amount of UV filters bound to proteins in human tissues. This involved treating the modified proteins with high levels of glutathione at pH 9.5. UV filters are released under these mild basic conditions and are trapped as GSH adducts that can then be quantified by HPLC. Using this methodology we showed that all normal lenses above the age of 50 have significant levels of UV filters bound to their proteins. Indeed the levels of bound are roughly equivalent to the free UV filter levels. This has major consequences. In collaborative work (with M. Davies, Heart Research Institute and J. Jamie, Macquarie University) we have also shown that when such modified proteins are illuminated by the wavelengths of light that pass the cornea, the proteins become oxidized. This may have relevance to the etiology of nuclear cataract. Thus the lenses of old people may be much more sensitive to the effects of light exposure than those of youngsters.

We are collaborating with researchers in the USA (Prof Frank Giblin) to determine the structures of novel UV filters that are found in the lenses of squirrels. Squirrels and humans have very similar UV filters in their lenses and they therefore may be a useful model for investigating the effect of oxidation and UV light on cataract formation.

**Posttranslational modification (PTM) in age-related cataract**
Korlimbinis, Truscott, Hains

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. Using this technique we have mapped all lens crystallins from older normal and cataract human lenses for the site and amount of deamidation. This PTM is one of the most abundant changes in old proteins and may lead to significant unfolding of the lens proteins.

**Ageing of human lens (Presbyopia)**
Freidrich, Truscott

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 7Murea. 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**
Friedrich, Truscott

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 recognized as the basis for understanding nuclear cataract. Ongoing studies into human lenses from the Lions Eye Bank are 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.

A collaboration with Professor Kevin Schey (Medical University of South Carolina) is underway using new iTRAQ methodology to quantify the binding of proteins to the membranes of lenses at the barrier region.

![The proteomics laboratory in action at the SSI](image-url)
Analysis of lens membrane components as a function of age
Deeley (Uni Wollongong), Truscott, Nealon (Uni Wollongong), Mitchell (Uni Wollongong), Stephen Blanksby (Uni 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 such changes may 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 relating these to separate measures of lens stiffness obtained by Dynamic Mechanical Analysis. We have shown that the membrane lipids of animals are quite different from those of humans. Human lens membranes also contain novel lipids that we have identified. In the next phase of research we are investigating how membrane changes influence the binding of denatured proteins – an important step in barrier formation.

Projects in 2009
Research in 2009 was carried out at the National Vision Research Institute at the Australian College of Optometry and University of Melbourne. The Visual Neuroscience Research Group moved to the Save Sight Institute in January 2010.

Structure of normal and myopic retina
Abbott, McBrien (Uni Melbourne), Grünert, Planta (Uni Melbourne)

The long-term aim of this project is to understand how the eye is changed by severe myopia (short-sightedness). We investigated the retinal origin of the optical coherence tomography (OCT) signal by aligning images taken from a live retina with corresponding vertical histologic sections. These results provide a basis for relating the OCT signal to underlying retinal anatomy. This interpretation can be used in the clinical setting to identify layers of the retina affected by disease such as myopia.

Synaptic inputs onto small bistratified (blue-ON / yellow-OFF) ganglion cells in the retina
Percival, Jusuf, Martin, Grünert

The retina is composed of ten different layers, and the inner plexiform layer, a dense layer of connections between different types of cells, contains subdivisions that segregate ON and OFF type light responses. We studied the ON and OFF connections to a class of retinal ganglion cells (small bistratified – blue-ON/yellow-OFF) ganglion cells in marmosets (Callithrix jacchus). The results show that direct input from bipolar neurons can account for both ON and OFF responses in this cell type.

Segregation of short-wavelength sensitive (S) cone signals in the brain
Roy (Uni Melbourne), Jayakumar (Uni Melbourne), Martin, Dreher (Uni Sydney), Saalmann (Uni Melbourne), Hu (Uni Melbourne), Vidyasagar (Uni Melbourne)

An important problem in the study of the mammalian visual system is whether retinal ganglion cells of different types are segregated anatomically in the brain. We recorded signals from individual cells in the LGN followed by histological reconstruction to investigate the distribution of colour-selective cells in the LGN of the macaque (Old World monkeys). We found that cells carrying signals from blue-cones were located in the koniocellular layers, while the red/green cells were found in the parvocellular layers. We conclude that there is anatomical segregation of blue/yellow and red/green colour signals for colour vision at early levels of visual processing in the brain.

Visual Neuroscience Research Group

Group Members
Paul R Martin BSc, PhD, Group Leader
Ulrike Grünert Dipl Biol, PhD, Group Leader
Carla Abbott BOptom, PhD, Research Officer
Sander Pietersen, Research Officer
Cindy Guy, Research Assistant
Kenny Cheong BBioMedSc (Hons), Postgraduate Student
Kumiko Percival BASc (Hons), Postgraduate Student

Research Activity
Our research aims to better understand the way visual information is transferred from the eye to the brain in segregated pathways for colour, motion and shape. We know that the image captured by the eye is sent to the brain as a series of parallel “movies” but the way that nerve cells (neurons) in the eye are wired together to create these movies is poorly understood. By analyzing the wiring diagram of the normal eye, we gain knowledge that can be used in clinical practice and treatment of eye disease.

Old proteins
Hooi, Friedrich, Lyons, Truscott

Old proteins are present at many sites in the body. We are investigating the changes that occur to these using a combination of accelerator mass spectrometry (at ANSTO) with sophisticated mass spectrometry approaches and HPLC to monitor the extent of amino acid racemisation.

Save Sight Institute Research Report 2009