Save Sight Institute Research Report 2006

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*
Michael Friedrich, *Postgraduate Student (Uni Wollongong)*
Karl Heys, *Postgraduate Student (Uni Wollongong)*
Jane Deeley, *Postgraduate Student (Uni Wollongong)*
Jasminka Mizdrak, *Postgraduate Student (Macquarie Uni)*

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 2006

UV filter Binding to Lens Proteins with Age
Korlimbinis, Mizdrak, 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.

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 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
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.

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

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. We have shown that the membrane lipids of animals are quite different from those of humans.

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 FRANZCO FRACS, *Clinical Associate Professor*
John Males, MBBS BSc FRANZCO, *Associate Lecturer*
Li Wen, MBBS MMed, *Research Officer*
Raj Devasahayam, BAppSc, *Eye Bank Laboratory Manager*
Meidong Zhu, MBBS MMed PhD, *Eye Bank Senior Hospital Scientist*
Mitchell Lawlor, MBBS, MMed(OphthSci), *Postgraduate 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 a 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 2006

Human herpes simplex virus (HSV) and herpes zoster virus
McClellan, Petsoglou, Wen.

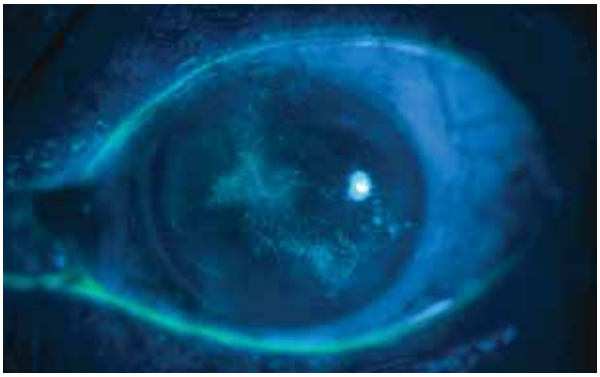
The group is gaining significant expertise in the techniques of PCR analysis (real time and in-situ) and immunohistochemistry for the human herpes virus. Ongoing projects include the distribution and form of these viruses in healthy human trigeminal ganglia and corresponding corneas, the genotyping of 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, Wen.

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, Petsoglou.

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.



Superficial keratitis plus dry eye

Infectious keratitis and confocal microscopy
McClellan, Males, Petsoglou, Devasayaham, Wen.

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. The Save Sight Institute has also developed significant expertise in the use of corneal confocal microscopy for the diagnosis of corneal infections. Purchased by the Sydney Eye Hospital Foundation the machine is able to image the cornea in patients with disease. It is invaluable in the diagnosis of fungal and protozoal infections of the cornea. Our results have been presented at a number of local meetings summarizing the groups experience. The Save Sight Institute also has patient's referred to it to diagnose corneal disease in the community.

UV light Collagen Cross Linking and Keratoconus
McClellan, Males, Petsoglou.

Keratoconus is the most common cause of vision loss in young people. It causes a slow sagging of the cornea resulting in distortion to a patients vision. Most patients can have this corrected by glasses or contact lenses, but 5% of all keratoconus patients will require a corneal transplant. There are currently no treatments for the underlying cause of the disease. The Sydney Eye Hospital and Save Sight Insiitute have set up an ethics committee approved trial for the use of Riboflavin eye drops (Vitamin B2) and UV light to prevent the progression of keratoconus. This follows on from overseas research demonstrating stabilization of patients corneas and in some cases reversal of the disease. The treatments results in bonds forming between the proteins in the cornea and thus stiffening its structure. The project has enrolled 30 patients and intial results are very encouraging. They were presented at the 2006 Cornea and Eye Bank Meeting in Auckland.

Human corneal endothelial cell culture
McClellan, Petsoglou, Wen, Zhu

In vivo, corneal endothelial cells do not normally replicate. They are essential in the maintenance of corneal clarity and vision. This recent project aims to define the conditions where human corneal endothelial cells can replicate in vitro, Drs Li Wen and Meidong Zhu have developed significant experience in these techniques and we have presented our preliminary results at the 2006 Cornea and Eye Bank meeting. 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.

Keratoconus
Sutton, McAvoy, Madigan, Green

In a collaborative study between the Save Sight Institute and Auckland University, original research is underway into the cause of keratoconus. Keratoconus is the major indication for corneal transplantation in Australia and affects 1 in 2000 in the population. The cross pollination of ideas across the Tasman has resulted in a unique and original approach looking at the basic metabolic problem in this condition. If our theory proves to be correct it could indicate possible therapeutic intervention

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 2006

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, *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
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 2006

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 Liason 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.

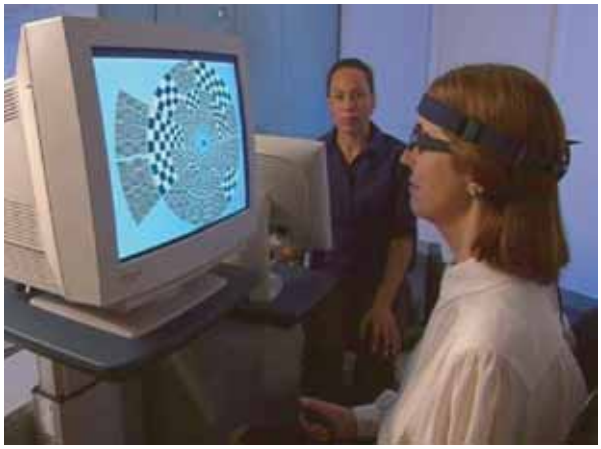
This research investigates the use of blue-yellow stimuli for presenting the multifocal Visual Evoked Potential (mVEP) in an attempt to detect glaucoma at an earlier stage. The rationale for this is that in subjective perimetry blue/yellow testing has been shown to be more effective at detecting early 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 then 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



Accumap system

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.

Eye Cancer Research Group

Michele Madigan, BOptom PhD, *Unit Co-Head (Basic Science)*
R Max Conway, PhD FRANZCO, *Unit Co-Head (Clinical and translational research)*
Frank Billson, MBBS FRANZCO FRACS FAC FRCOphth
Yong Li, BSc PhD
Vidushi Sharma, MBBS, *Paediatric Fellow*
Thomas Cunneen, MBBS MMed, *Postgraduate Student*
Kenneth Lai, BMedSci, *Postgraduate Student*
JingJing You, *Honours Student (co-supervised with Oncology Research Centre, UNSW)*

Research Activity

Better understanding the normal processes of development and ageing (including UV damage by the sun's rays) in the retina and eye provides insight into the pathogenesis of primary eye tumours. In particular, 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. 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 chemotherapy, and the high incidence of untreatable metastases in ocular melanoma.

Effects of biological response modifiers on growth of eye melanomas
Madigan, Conway, Cuneen

These studies are investigating the efficacy of various biologic response modifiers such as retinoids, histone deacetylase inhibitors and interferons in controlling intraocular, ocular surface and metastatic tumour growth. These agents have very low toxicity compared to standard chemotherapy and can control tumour growth by inducing tumour cell differentiation. 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 techniques developed in these studies are also being applied to studies of prostate 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).

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^2). 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.

In vivo effects of retinoids and beta-laprochone 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-laprachone 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 potentially reduce the dose of cytotoxic therapies required when treating tumours.

The expression and distribution of MUC18 in uveal melanoma
Lai, Sharma, Conway, Jager (Leiden) and 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 oberavtions in primary tumours also indicate interactions between MUC18-positive melanoma cells and vasculature that may be important for the hematogenous spread of tumor cells during metastases.

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 FRCOphth
Chris Willcock, BSc(Hons), *Research Assistant*
Marija Mihelec, BSc(Hons), *Postgraduate Student (CMRI)*
Luke St Heaps, BSc(Hons), *P/T Postgraduate Student (CHW)*
Peter Abraham, *Honours Student (Macquarie Uni)*

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).

Projects in 2006

The genetic basis of microphthalmia and anophthalmia
Jamieson, Grigg, Billson, Willcock, Mihelec, Abraham

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. We have identified the deletion of several genes including OTX2 and BMP4 in a patient with bilateral anophthalmia. We are proceeding with the analysis of novel candidate disease genes in another chromosomal translocation patient. Genetic analysis is also proceeding in a large family with several affected members with microphthalmia and anophthalmia.

Disease gene studies in cataract, glaucoma and anterior segment dysgenesis
Mihelec, St Heaps, Willcock, Grigg, Billson, Jamieson

Some genes are known to be associated with anterior segment dysgenesis, including PAX6 which is particularly implicated in aniridia. We have shown the unusual phenotype of ectropion uveae to be associated

with PAX6 mutation. In this work we are establishing a protocol for analysis of the PAX6 gene for use in future clinical situations. We are also analysing chromosomal translocation breakpoints for novel candidate disease genes in cataract and glaucoma.

Genetics of macular dystrophy
Jamieson, Abraham, Grigg, Billson, Mihelec

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. In our investigation of these families we aim to identify a new process critical to the health of the macula.

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

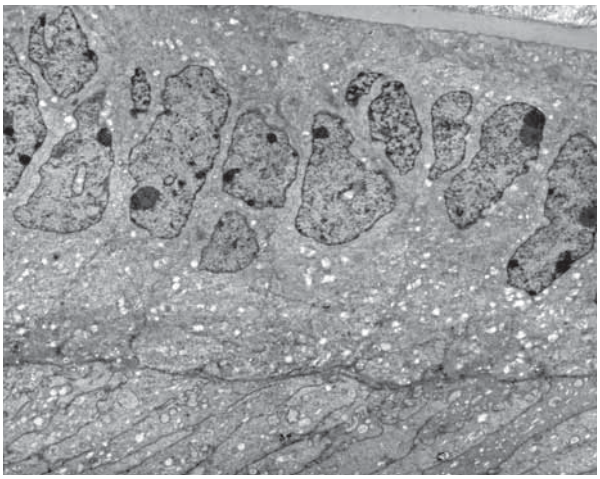
John McAvoy, BSc PhD, *Unit Co-Head*
Frank Lovicu, BSc PhD, *Unit Co-Head*
Richard Stump, BSc PhD, *Laboratory Manager*
Jessica Boros, BSc, *Research Assistant*
Sharon Ang, BSc, *Research Assistant*
laxmi Iyengar, BSc, *Postgraduate Student*
Yongjuan Chen, BSc, *Postgraduate Student*
Colin Chong, MBBS,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



Lens epithelium - electron microscope image

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 (TGFß) 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 TGFß 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 2006

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
McAvoy, O'Connor (British Columbia Cancer Research Centre), 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 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/ß-catenin and Planar Cell Polarity 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, 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 TGFß-induced cataract
Chong, Ang, Lovicu, McAvoy.

Transforming growth factor-beta (TGFß) 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 TGFß-induced cataract models. We showed that Wnt expression is altered in TGFß-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 TGFß-induced cataracts and may play a role in human cataract, such as posterior capsule opacification.

Lithium stabilises the polarised lens epithelial phenotype and blocks TGFß-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/ß-catenin signalling in lens epithelial differentiation, we assessed the effects of lithium chloride (LiCl), which is often used to mimic aspects of ß-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, ß-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 ß-catenin signalling. By contrast, in polarised LiCl-treated cells, ß-catenin remained localised to cell margins and, as in vivo, there was no evidence of ß-catenin signalling. Significantly, the effects of lithium also extended to blocking the cataract-promoting effects of TGFß 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.

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/nidigen. 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 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

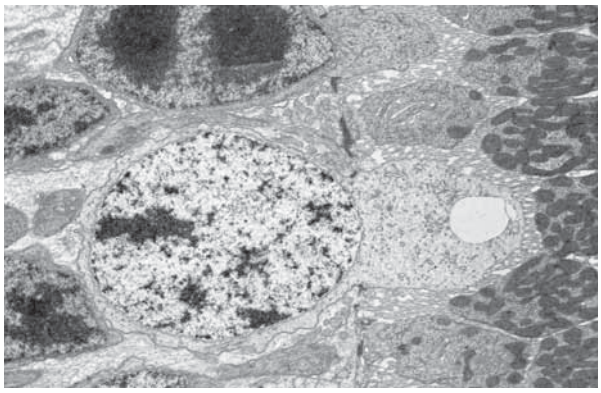
Jonathan Stone, BSc PhD FAA, *SSI Associate (ANU), Unit Head*
Diana van Driel, BSc(Hons), *Senior Research Assistant*

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.



Wallaby photoreceptors - electron microscope image

Projects in 2006

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

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*
Elisa E Cornish, BMedSci PhD, *Research Assistant (P/T)*
Alexandra Allende, MBBS, *Postgraduate Student*
Phillip Romo, *Honours Student*
Elizabeth Shelley, *Honours Student (ANU, supervised by Dr Jan Provis)*

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.

Projects in 2006

Photoreceptor degeneration in normal ageing and age-related macular degeneration
Shelley (ANU), 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. Using DNA sequencing, we will identify polymorphisms for genes important for innate immunity (such as Complement Factor H). These studies are important for understanding whether low-grade inflammation, combined with these underlying gene alterations, are important for the pathogenesis of AMD.

Blood vessel growth during foveal development
Romo, 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 TGFß 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:
Maria Williams, RN BN BA GDip (Acute Care Nurs) *Clinical Research Officer*
Haipha Ali, BSc (Applied Vision Sciences, Orthoptics), *Refractionist and Photographer*
Christine Gaston, MBBS, *Clinical Research Officer*
Meidong Zhu, MD PhD, *Senior Research Fellow*
Nicky Cunningham, *Clinical Research Officer*
Ramachandran Unnikrishnan Nair, *Visiting Retinal Fellow*
Rajeev Jain, *Visiting Retinal Fellow*
Grace Hunt, MBBS, *Photographer*
Laboratory Research Unit:
Martin Windsor, BSc PhD, *Senior Research Fellow*
Alice Len, PhD, *Research Fellow*
Bryony Stracey, *Technical Officer*
Svetlana Cherepanoff, BMedSci MBBS, *Postgraduate Student*
Goff Quin, MBBS, *Postgraduate Student*
Marina Tretiach, BAppSc PhD, *Postdoctoral Student*
Jenny Wyndham, BSc DipEd MS MPH, *Postgraduate Student*
Xin Yuan Zhang, *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 four investigator initiated clinical trials were undertaken in the Unit in 2006. 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 telangectasia.

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 2006

Clinical Projects

Open label extension of a clinical trial of intravitreal triamcinolone for diabetic macular oedema (TDMX Study)
Gillies, Zhu, Ali, Williams, Gaston, Nair, Jain, 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. 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. A prospective, double-masked, placebo-controlled randomised clinical trial that investigated the efficacy and safety of intravitreal injections of triamcinolone acetonide for diabetic macular oedema that had failed laser treatment was conducted between February 2002 and April 2005 . 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. 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. This study is a three year open-label extension of the original study in which all eyes receive active treatment, including those that previously received placebo. The study is supported by an NHMRC project grant for 2006-2008.

Chorioretinal venous anastomosis for non-ischaemic central retinal vein occlusion
McAllister (Uni WA), Gillies, Mitchell (Westmead), 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 developed. The current project is an NHMRC-funded 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 and completed the study at our site. The data analysis is ongoing in the Lions Eye Institute, Perth.

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

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 how treatmentwith intravitreal triamcinolone, which has been pioneered by the Save Sight Institute, is best combined with conventional laser photocoagulation for the treatment of 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.

Seventy five eyes of 49 participants had been enrolled into the study by the end of 2006 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.

A natural history study of macular telangiectasia (The MacTel study)
Gillies, Zhu, Ali, Gaston, Williams, Cunningham, Hunt.
This natural history study, sponsored by the Lowy Medical Research Foundation, is aiming to characterise the clinical features of type IIa idiopathic perifoveal 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 three 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 6 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. There were 16 patients who were enrolled into the study in 2006. These brought the total number of 22 participants in our study site and put our site at the third position of 25 sites around the world. Patient enrollment is still continuing. Associate Professor Mark Gillies was appointed Executive Scientific Director of the entire project in 2006.

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, Pasadhika.

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.

Reduction in occurrence of centre-threatening diabetic macular oedema. Protocol B7A/MC/MBDL
Sponsored by Eli Lilly Pharmaceuticals
Gillies, Williams, Zhu, Gaston, Ali.

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 were enrolled into the study in 2006.

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.

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.

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 intravitreous 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, Pasadhika.

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.

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 700_g and 350_g 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.

This 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

In vitro modeling of the Blood-Retinal barrier
Canning

The aim is to establish a reliable in vitro bioassay to model the BRB, and test by proteomic analysis any candidate proteins that may improve barrier function.

Human retinal Müller cells are isolated from post-mortem eyes and their proteins are compared with those from astrocytes in age-matched patients in order to establish unique features of retinal Müller cells. This may elucidate the mechanisms by which Müller cells contribute to BRB integrity and also shed light on the pathogenesis of macular telangiectasia.

Autoantibodies in macular degeneration
Cherepanoff, Gillies.

Antibodies directed against retina are known to occur in age-related macular degeneration. This project is to

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.

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.

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.

In vivo analysis of the blood retinal barrier (BRB) in diabetic rats
Windsor, Gillies

Studies were undertaken to develop ways to model diseases, such as diabetic retinopathy, that cause swelling of the retina through breakdown of the blood-retinal barrier. Techniques under evaluation are tracer-based, for example Evans Blue dye or fluoresceinylated albumin. It is intended that this model will be used to screen for treatments of diabetic retinopathy.

Triamcinolone Acetonide Inhibits Diabetic Blood Retinal Breakdown
Zhang, Gillies

Vascular endothelial growth factor-A (VEGF-A)/vascular permeability factor is believed to make blood vessels grow and leak abnormally in major retinal diseases such as diabetic retinopathy and age-related macular degeneration. VEGF-A works by activating two cell surface receptors, FLT-1,and FLK-1 Inhibition of VEGF over-expression is thus a potential treatment for diabetic retinopathy.

Triamcinolone acetonide is long acting steroid. In our two year clinical trial, eyes with swelling of the retina caused by diabetes that had failed conventional laser treatment that were treated with an injection of into the eye of triamcinolone had twice the chance of improving vision and half the risk of further loss. However, a high incidence of cataract and elevated eye pressure was found in the treated eye.

To find out how intravitreal steroid therapy works on on diabetic retinopathy, we investigated the effect of injections into the eye of triamcinolone on the levels of VEGF-A and its receptors in diabetic rat retinas. We then correlated the expression of these proteins with breakdown of the blood-retinal barrier.

Save Sight Institute staff