Save Sight Institute Research Report 2008

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

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Michael Friedrich, Postgraduate Student (Uni Wollongong)
Jane Deeley, Postgraduate Student (Uni Wollongong)
Brian Lyons, Postgraduate Student
Jessica Nealon, Postgraduate Student (Uni Wollongong)
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Michelle Hooi, Postgraduate Student

Research Activity

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

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

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

Projects in 2008

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.

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.

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 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) is underway using new iTRAQ methodology 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, Nealon, with Todd Mitchell, Stephen 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 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.

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

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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
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 469 penetrating corneal grafts to the patients of NSW in 2008. 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 2008

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. 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.

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

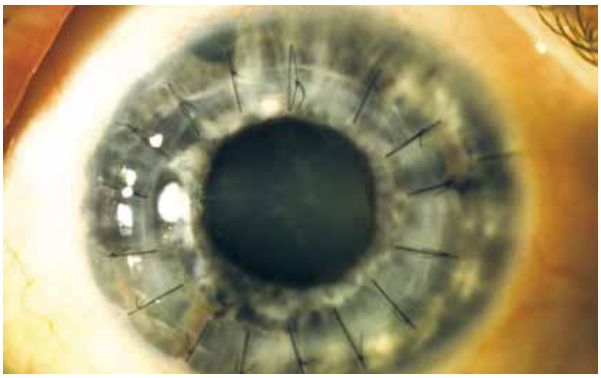
The development of an optimised organ culture medium for corneal grafts is being actively researched. This involves the investigation of corneal metabolism during storage and the viability of endothelial cells after transplantation.

Utility of corneas from donors with previous intraocular surgery
Zhu, Devasayaham, Georges, McKeon, McClellan, Petsoglou

From statistical data collected by the Lions NSW Eye Bank 8.3% of eyes retrieved could not be used for transplantation due to previous intra-ocular surgery. This figure is increased to approximately 10% when donors were ruled out due to previous ocular surgery and were not retrieved. This raises the issue of whether

the donors with previous intra-ocular surgery could be included for penetrating keratoplasty transplantation. Although previous intraocular surgery is no longer a contraindication for eye donation according to the Protocol of the Eye Bank Association of Australia and New Zealand in 2005 and all Eye Banks in Australia and New Zealand accept these corneas, there is very little information and little published clinical data about the qualities of these corneas used for operation for the donors, recipients and ophthalmologists.

The Lions NSW Eye Bank is conducting a study, with the support of Lions NSW-ACT Save Sight Foundation, to determine whether these donor eyes with previous intra-ocular 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 the quality information about the corneas from eyes that had previous ocular surgery.



Corneal transplant

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 group's experience. The Save Sight Institute also has patients 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 patient's 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 Institute 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 patient's corneas and in some cases reversal of the disease. The treatment's results in bonds forming between the proteins in the cornea and thus stiffening its structure. The project has enrolled 30 patients and initial 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 2007 Cornea and Eye Bank meeting in New Zealand. 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 cell in corneal reconstruction
Zhu, Wen, Petsoglou, 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 cell to be repaired or regenerated. Currently, the group has successfully achieved corneal epithelial cells from bone marrow derived MSCs. The research results had been presented in the 2008 XVIII International Congress for Eye Research in Beijing. A paper is in preparation.

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

Keratoconus (KC) is a bilateral progressive, noninflammatory degeneration of the cornea, associated with decreasing visual function. Visual function is lost related 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 diagnostic and therapeutic application in modulation of the Wnt pathway for the treatment of keratoconus. Whilst the research is in its infancy, the group has presented internationally and submitted for publication novel findings in the pathogenesis of keratoconus.

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 2008

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 practicing 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 2008. 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

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Alessandra Martins MBBS, Postgraduate Student
Chandra Balachandran MBBS, Postgraduate Student
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Asya Klistorner, Electrophysiology Technician
Dr Maria Korsokova, 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 2008

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

The Save Sight Institute continues to work in collaboration with ObjectiVision Pty Ltd to develop the AccuMap V2 objective perimeter as a method for investigating visual function particularly in glaucoma. The AccuMap V1.3 was successfully launched in Australia, but despite FDA approval was not widely adopted in the USA. We have therefore sought to address some of the limitations of the system and improve its diagnostic performance to make it a potential benchmark for objective detection of glaucoma. The version 2 system is in the final prototype phases, and will incorporate several major advances. Firstly, the original large CRT screen is replaced with a flat high speed LCD screen, and the computer is replaced with a laptop, both making the system much more compact and potentially portable. Secondly, a completely new electrode headset has been designed with a preamplifier located on the headset itself. This dramatically improves signal to noise ratios and will improve reliability of recording and shorten test times. Thirdly, a new disposable electrode cross has been developed which can be clipped onto the preamplifier for recording then discarded. This saves time for the clinician and removes hygiene issues associated with cleaning and re-use of the electrodes. The new system will also incorporate new software for calculating the signal latency, which is very important in optic neuritis (see project p6).

Development of a Binocular Multifocal Visual Evoked Potential using Virtual Reality Goggles
Klistorner, Graham, Srinivasan, Grigg

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

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

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

At present there are 5 US trial sites involved in the multicentre AccuMap Early Glaucoma Detection Study, with Dr Klistorner and Dr Graham at the Save Sight Institute being co-ordinators. The results of this study will help confirm the effectiveness of the technique compared to conventional methods of detecting glaucoma. Dr Grigg and Prof Billson are principle investigators.

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

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

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

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

Group Members

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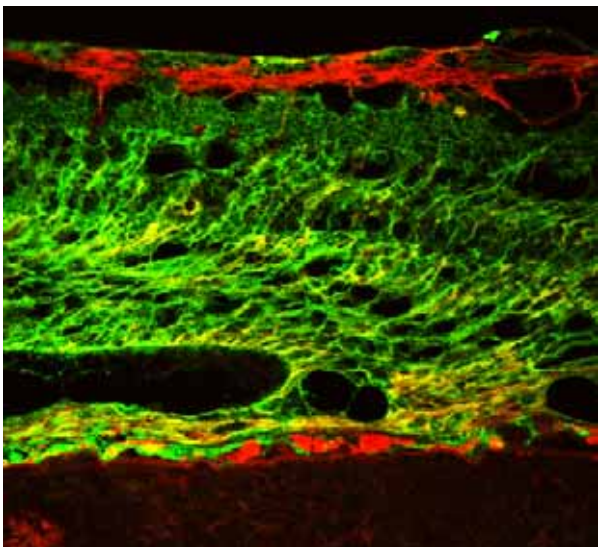
Natalie Walsh-Conway PhD, Research Associate
Yong Li BSc PhD, St George Hospital, UNSW
Kenneth Lai BMedSci, Postgraduate Student
Sarah Rahman BOptom, Honours Student (UNSW)
Jennifer Chow BOptom, Honours Student (UNSW)



Retina from eye with choroidal melanoma showing GFAP (red) and CRALBP (green) labelling; confocal image

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 2008

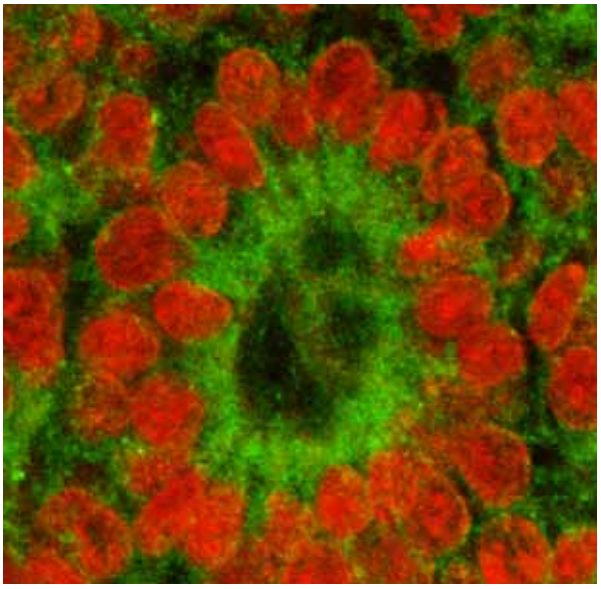
Effects of histone deacetylase inhibitors (biological response modifiers) on growth and viability of ocular melanomas
Madigan, Conway, Cuneen

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), Jager (Leiden Medical Centre), 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).



Retinoblastoma tumour cells showing FGFR4 labelling (green); cell nuclei are stained red; confocal image

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-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 reduce the dose of cytotoxic therapies required when treating tumours.

The expression and distribution of MUC18 in uveal melanoma
Lai, 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 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 FRCOphth
Rebecca Storen BSc, Research Assistant
Yongjuan Chen BSc PhD, Postdoctoral Fellow (CMRI)
Marija Mihelec BSc (Hons), Postgraduate Student (CMRI)
Luke St Heaps BSc (Hons), P/T Postgraduate Student (CHW)
Maja Popovic, Honours Student

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 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. 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 2008

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

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 large family with several affected family members, we have identified a novel mutation in the SOX2 gene. This mutation is in the DNA-binding partner interaction region of SOX2 and provides insight to the specification of the functions of SOX2 in eye development. In addition, our investigation of mouse models with microphthalmia, indicate a role for the regulation of Wnt signaling in eye development.

High-resolution genomic studies in cataract, glaucoma, anterior segment dysgenesis and retinal dystrophies
Mihelec, 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. High-resolution genomic analysis is also useful in conditions such as aniridia, which is known to be associated with mutations in the PAX6 gene. We have established a protocol for analysis of the PAX6 gene and this is now available for clinical use in Australia.

Genetics of macular dystrophy
Jamieson, Grigg, 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. A linkage region has been identified and we are investigating candidate genes in this region.

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
Cynthia Tang, Honours Student

Research Activity

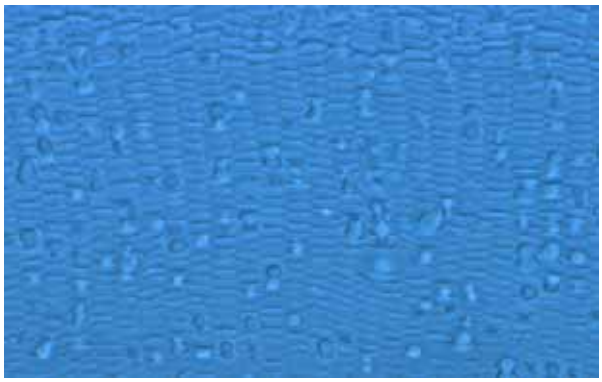
The lens transmits and focuses light onto the retina. To do this it needs to be transparent and to have appropriate refractive properties. This depends on the development and maintenance of highly ordered cellular architecture.

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

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

We have focused our attention on growth factors because of their importance in regulating cell fates in developmental systems. Using a unique lens epithelial explant culture system we have identified members of the FGF growth factor family as inducers of lens cell proliferation, migration and differentiation; responses that are induced in a progressive dose-dependent manner. 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.



Cross section of 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 2008

The role of FGF antagonists in lens biology and pathology
Shin, Boros, McAvoy, Tang, 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 normal lens polarity (ie 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, 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, 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 signaling 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 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
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 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, 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 expression of Wnts 5a, 5b, 7b, 8a and 8b are distinctly upregulated in cataractous

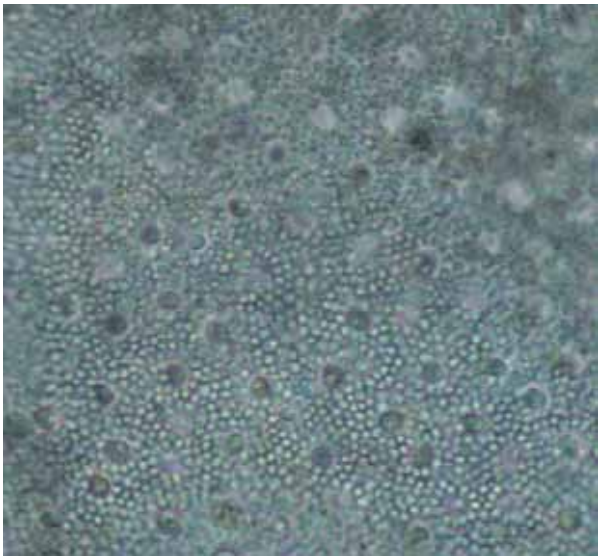
plaques of all the models we examined. This indicates that Wnt signalling is involved in regulating abnormal growth and differentiation processes in TGFß-induced cataracts and may play a role in human cataract, such as posterior capsule opacification

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, SSI 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



Human photoreceptors

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 2008

Current joint projects include the use of dietary anti-oxidants, 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
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)

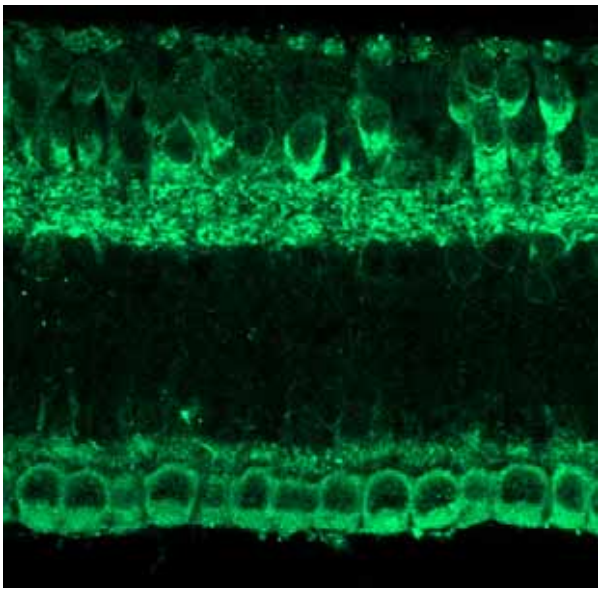
Research Activity

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 2008

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.



Developing primate retina showing doublecortin (green) labelling of photoreceptors and bipolar cells

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 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
Edward Hughes MBBS (Hons), Visiting Vitreoretinal Fellow
Andrew Lim MBBS (Hons), Visiting Vitreoretinal Fellow
Yun Chin Siow MBBS, Visiting Vitreoretinal Fellow
Grace Hunt MBBS, Photographer
Jacqueline Oh BAppVisSc, Orthoptist and Photographer
Briony Glastonbury MHSM, FRB! Project Manager
Ann Gould BSc, Clinical Research Officer
Liudmila Kolmogorova BMedSc, Administrative Assistant

Laboratory Research Unit:
Weiyong Shen MD PhD, Senior Research Fellow
Alice Len PhD, Research Fellow
Shiying Li MD PhD, Postdoctoral Research Fellow
Sook Hyun Chung BMedSc (Hons), Research Assistant
Robert Rapkins BSc (Hons), Research Assistant
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Svetlana Cherepanoff BMedSci MBBS, Postgraduate Student
Goff Quin MBBS, Postgraduate Student
Xin Yuan Zhang, Postgraduate student
Narelle Jay BBiotech (Hons), Postgraduate student
Yun Ching Siow MBBS, Visiting Retinal Fellow
Liudmila Kolmogorova BMedSc, Administrative Assistant

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. The trials the unit undertakes. involves 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 telangiectasis. 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 2008

Clinical Projects

Open label extension of a clinical trial of intravitreal triamcinolone for diabetic macular oedema (TDMX Study)
Gillies, Zhu, Ali, Williams, Gaston, Siow, Hunt, Hughes, Lim, 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 the TDMX study, the open label extension of TDMO. The trial began progressively from the 24 month visit of TDMO study for each patient. We have now completed a three year TDMX follow up in which all the eyes of study participants were treated with medication (intravitreal triamcinolone) as required, as well as standard laser treatment where appropriate. Analysis of results from both studies combined showed improvement in vision (of approximately one line on the vision chart after a total of 5 years) in 14/33 (42%) eyes initially treated with triamcinolone compared with 11/34 (32%) eyes initially treated with placebo. 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. A paper reporting the 5-year results is currently in preparation.

A multicentre randomised clinical trial of laser treatment plus intravitreal triamcinolone for diabetic macular oedema (Thunderbird study)
Gillies, Zhu, Ali, Williams, Gaston, Cunningham, Hunt, McGimpsey, Siow 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 treatment with 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 in both 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 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 eighty-four eyes of 54 participants enrolled into the study by the end of April, 2007 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. Thirty-eight participants (62 eyes) completed 2-year follow-up by the end of 2008. Eight participants (10 eyes) withdrew due to either patients passed away or were unable to attend clinics by the end of 2008. The abstract of 6-month study results was accepted for presentation at the 2009 Annual Meeting of The Association for Research in Vision and Ophthalmology, in USA. The results suggest that despite a better anatomical outcome reflected by reduction in mean central macular thickness, visual results and the need for further laser treatment at 6 months were no better in the IVTA-treated group. IVTA was associated with a significant IOP rise. The final results will be available by mid 2009.

A natural history study of macular telangiectasia Type 2 (The MacTel study)
Gillies, Zhu, Ali, Gaston, Williams, Hunt, Oh

This natural history study, is aiming to characterise the clinical features of macular telangiectasia type 2 (MacTel Type 2) 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) participate in a family history/genetics sub-study. The study is sponsored by the Lowy Medical Research Foundation and the Executive Scientific Director is Professor Mark Gillies.

Total three hundred and eighty subjects were enrolled in the study by the end of 2008 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. The participants are seen at the Sydney Eye Hospital every 12 months for testing of best-corrected visual acuity, relevant eye examinations and blood tests and are followed up by telephone every other 6 months to track adverse events. The study is for at least five years. Our site enrolled the first patient in the world in October 2005 and had 36 participants by the end of 2008. This puts our site in second position of 25 sites around the world. In addition to the MacTel patient enrolment, our centre also screened 56 first degree family members and accrued 13 control subjects (of a total of 148 family members and 38 controls in all 25 sites) for the MacTel Genetics study by the end of 2008. This puts us at number one position in the world for the third 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 any disturbance of vision (i.e. currently all the participants in the Natural History Study) have an advanced form of the disease. A paper of the research results from the family member study is in preparation. Patient enrolment and family member screens are still continuing.

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

This three year multicentre, international placebo controlled trial of an oral protein kinase C beta isoform inhibitor for patients with diabetic macular oedema was commenced in 2004 and final patient visits were completed in 2008 as scheduled.

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, Siow

This 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. This study was completed in 2008.

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

This trial commenced in 2005. Patients with central and branch retinal vein occlusion received a slow release pellet of dexamethasone, inserted in the eye (or sham procedure) at baseline and were followed for six months. At six months all patients received an active dexamethasone pellet and were followed for another six months. In total 21 patients were enrolled over the course of this study and final patient visits were completed in 2008.

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
Researcher: Gillies M, Zhu, Ali, Gaston, Siow

This trial commenced in March 2007 and 6 patients were enrolled. All final patient study visits were completed in 2008.

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, Gould, 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 are enrolled and study completion is scheduled for 2009.

The efficacy and Safety of Treatment with Intravitreal Ranibizumab in Patients with Branch Retinal Vein Occlusion 'L-BRVO'
Sponsored by Lions Eye Institute (WA); Study Drug supplied by Novartis
Gillies, Gaston, Williams, Gould, Zhu, Ali, Hughes, Lim

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. It is envisaged the study would be completed in 2010.

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')
Sponsored by Novartis
Gillies, Gaston, Gould, Zhu, Ali, Lim

This multicentre international trial in patients with diabetic macular oedema involves patients being randomized to one of three treatment arms: 1) ranibizumab intravitreal injections, 2) ranibizumab intravitreal injections plus laser and 3) laser alone (1:1:1). Patient participation is over 12 months. This trial commenced in late 2008 with recruitment ongoing.

Fight Retinal Blindness!
Funded by The Eye Foundation
Gillies, Wong (Uni Melb), McAllister (Uni WA), Gaston, Glastonbury

The Fight Retinal Blindness! Project is a collaborative initiative between the Save Sight Institute, Sydney; Retinal Vascular Imaging Centre, Melbourne; and Lions Eye Institute, Perth. The overall goal of the project is to develop strategies to reduce the incidence of retinal blindness in the Australian community.

The Project will:
1. Monitor, track and evaluate new treatment strategies for MD 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;
2. Undertake linkage of data across population health databases to inform assessment of morbidity and mortality associated with the new treatments for macular disease; and
3. Develop broader strategies to reduce the incidence of retinal and other blindness in Australia.

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.

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.

Glia-vascular dysfunction in perimacular telangiectasis type 2
Shen, Li, Chung and Gillies

Macular telangiectasis (McT) type 2 is an uncommon but potentially blinding condition of the retina. The predominant and most consistent clinical features in this retinal disease include loss of retinal transparency, intra-retinal vascular abnormalities, including small telangiectatic vessels and vascular leakage, disturbance to photoreceptors and inner and outer lamellar retinal cavitation. Currently, the causes of McT are entirely unknown. Müller cells span the entire thickness of the retina and ensheath all retinal neurons. This morphological relationship reflects a multitude of functional interactions between Müller cells-retinal vasculature and Müller cells-retinal neurons. Müller cells are involved in regulation of the blood retinal barrier (BRB) and participate in the control of retinal angiogenesis. This study aims to determine the effect of retinal glial dysfunction on retinal vascular changes in rodents and in non-human primates.

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-aminoadipic acid (DL-alpha-AAA) and siRNA specifically targeting glutamine synthetase in diabetic rats. The main purpose of this study is to determine whether further disturbance of retinal glial function exacerbates DR. Retinopathy in chemically induced diabetic models otherwise has a very mild phenotype.

Generation of an inducible transgenic model for Müller cell-specific genetic targeting
Shen, Fruttiger, Chung 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 ($CreER^{T2}$). 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-CreER 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 4 days after tamoxifen induction in adult animals (6 weeks old). The LacZ expression was specifically localised to Müller cells in the retina. The number of LacZ expressing Müller cells increased after multiple tamoxifen administrations (3x) compared with one dose induction. LacZ expression was not observed in the retinal pigment epithelium and other types of retinal cells. Results from our preliminary studies indicate that regulatory elements in a restricted region of the Rlbp1 gene are sufficient to drive transgene expression in a Müller cell-specific manner in the retina in vivo. This transgenic line may provide a useful tool for future studies of the role of Müller cells in retinal diseases.

Role of bone marrow derived progenitor cells in diabetic retinopathy
Shen, Li, 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 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.

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.

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.

Effects of Triamcinolone Acetonide on Diabetic Retinopathy
Zhang, Gillies

Diabetic retinopathy (DR) is the most prevalent and severe complication of diabetes. Triamcinolone acetonide is a 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, the mechanism of the action of the medicine is still uncertain.

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.

It has been well established that neural degeneration occurs in DR. Steroid treatment has been proved to protect neurons in other diseases, but little is known about the possible mechanisms.

To find out how intravitreal steroid therapy works on diabetic retinopathy, we investigated the effect of injections into the eye of triamcinolone on the levels of VEGF-A and its receptors, we then correlated the expression of these proteins with breakdown of the blood-retinal barrier; furthermore, we evaluated the neuro-protective effects of triamcinolone in a rodent model of early diabetic retinopathy.

Wnt signaling in diabetic retinopathy
Rapkins, 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.

In studies initiated by our 2002 ORIA grant, we identified significantly elevated levels of β-catenin in the cytosolic fraction of diabetic rat retina. To date there have been no studies reported that have examined β-catenin or Wnt signalling in the pathogenesis of DR or any of the other eye complications of diabetes.

To further investigate the role of β-catenin and Wnt signalling in DR we have employed the use of a Wnt focussed PCR array in diabetic mice. Preliminary data has revealed that a range of Wnt signalling genes are up- or down-regulated in DR. Moreover, we have been able to isolate the precise cell-type in which a number of these genes have displayed varied express patterns by immunohistochemistry.

Future investigations will focus on determining the precise Wnt pathway(s) affected by DR in the hope of better understanding the mechanisms that underlie the aetiology of this debilitating disease, with the prospect of providing more focussed drug targets.

Some of the Save Sight Institute staff