2005 Research Abstracts

A compilation of investigations made by Cleveland Clinic Cole Eye Institute physicians, research scientists and distinguished colleagues. The abstracts presented were published in selected journals or presented at selected national meetings in 2005.

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Clinical and basic ophthalmology research at the Cole Eye Institute is carried out by our professional staff of 60 physicians and scientists, who offer expertise in every ophthalmic subspecialty area.

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Sincerely,

Hilel Lewis, M.D.
Director, Cole Eye Institute
Chairman, Division of Ophthalmology
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Cole Eye Institute Staff

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Section 1

Cornea
A Device to Facilitate Limbal Stem Cell Procurement from Eye Bank Donor Tissue for Keratolimbal Allograft Procedures

David M Meisler, Victor L Perez, J Proudfit

**Purpose:** To develop a device that facilitates the procurement of corneal limbal stem cell grafts for keratolimbal allograft procedures used in the treatment of ocular surface disease associated with stem cell deficiency.

**Design:** Description of device design and technique for use.

**Methods:** The device is composed of a pedestal with a convex surface mounted to a flat platform. A corneoscleral button placed endothelial side down and centrally upon the convexity is secured by suction conveyed through a hollowed core in the pedestal that connects to fenestrated openings on the convex surface. A donut-shaped stainless steel ring placed on tension by springs braces the peripheral tissue. A circular corneal incision is created of a desired thickness by a suction trephine, and a crescent blade is utilized to peripherally dissect a donut-shaped keratolimbal allograft.

**Results:** This device facilitated the harvesting of the keratolimbal allograft tissue from four eye bank donor practice corneoscleral buttons and was then used to successfully procure grafts from six corneoscleral buttons used in three keratolimbal allograft procedures in three patients, one each with aniridia, alkali burn, and drug-induced limbal stem cell deficiency.

**Conclusions:** The described device effectively facilitates procurement of corneoscleral buttons for keratolimbal allograft procedures. It appears to offer advantages over freehanded techniques and previously described devices used for the same purpose.
An Evaluation of Image Quality and Accuracy of Eye Bank Measurement of Donor Cornea Endothelial Cell Density in the Specular Microscopy Ancillary Study

Cornea Donor Study Group

Purpose: The Specular Microscopy Ancillary Study was designed to examine donor corneal endothelial specular image quality, compare the central endothelial cell density determined by eye banks with the endothelial cell density determined by a central specular microscopy reading center, and evaluate donor factors that may have an impact on specular image quality and endothelial cell density accuracy.

Design: Nonrandomized comparative trial.

Participants: Endothelial specular images of donor corneas assigned in the Cornea Donor Study.

Methods: Certified readers assessed donor image quality (analyzable from fair to excellent vs. unanalyzable) and determined the central endothelial cell density. Independent adjudication was performed if there was a difference in the quality of grading or if the endothelial cell density varied by ≥5.0% between readers. Average reading center–determined endothelial cell density was compared with the endothelial cell density determined by each eye bank.

Main outcome measures: Evaluation of image quality and accuracy of endothelial cell density.

Results: Of 688 donor endothelial images submitted by 23 eye banks, 663 (96%) were analyzable (excellent, 40 [6%]; good, 302 [44%]; fair, 321 [47%]), and 25 (4%) were unanalyzable by reading center standards. In situ retrieval and greater epithelial exposure correlated with a higher image quality grading. The eye bank–determined endothelial cell density of 434 of the 663 (65%) analyzable images were within 10% of the endothelial cell density determined by the reading center, whereas 185 (28%) were more than 10% higher and 44 (7%) were more than 10% lower. Greater variation in endothelial cell density between the eye banks and the reading center was observed with shorter time of death to preservation, presence of an epithelial defect, folds in Descemet's membrane, lower image quality, and the use of fixed-frame or center method endothelial cell density analysis.

Conclusions: Overall, donor endothelial specular image quality and accuracy of endothelial cell density determination were good. However, the data suggest that factors that may affect image quality and contribute to variation in interpretation of the endothelial cell density should be addressed, because the donor endothelial cell density is an important parameter for assessing long-term corneal graft survival.
Bacterial Culture Isolates from Hospitalized Pediatric Patients with Conjunctivitis

Ahmad B Tarabishy, Gerri S Hall, Gary W Procop, Bennie H Jeng

**Purpose:** To determine the causative organisms of acute bacterial conjunctivitis in hospitalized pediatric patients at a tertiary care referral center.

**Methods:** A retrospective chart review was performed evaluating all pediatric patients hospitalized at the Children’s Hospital of the Cleveland Clinic Foundation from January 1, 1996, to November 30, 2004, who developed clinical signs of acute bacterial conjunctivitis (red eye with purulent discharge) and had culture-positive conjunctival swabs. The frequency of each bacteria and their susceptibility to antibacterial agents were recorded.

**Results:** One hundred seven bacterial isolates were cultured from eyes of 59 patients. A single organism was found in 27 (45.8%) patients. The most common organism was coagulase-negative *Staphylococcus*, found in 59.3% (35 of 59) of patients. *Viridans Streptococcus* sp. and *Staphylococcus aureus* were found in 47.5% (28 of 59) and 20.3% (12 of 59) of patients, respectively. Less common organisms included *Hemophilus influenzae* (17.0%), *Pseudomonas aeruginosa* (8.5%), and *Escherichia coli* (6.8%). Among the 42 isolates of coagulase-negative *Staphylococcus* identified, 26 were tested for antimicrobial susceptibility, and 65.3% (17 of 26) were methicillin-resistant. Similarly, 13 of 13 isolates of *Staphylococcus aureus* were tested for susceptibility, and 30.8% (4 of 13) were methicillin-resistant.

**Conclusions:** The distribution of bacterial organisms causing acute conjunctivitis in our hospitalized patients differs from that of previous reports of pediatric patients who present with acute bacterial conjunctivitis in an outpatient setting. In addition, there seems to be a high rate of methicillin-resistance among coagulase-negative *Staphylococcus* and *Staphylococcus aureus* isolates in our hospital setting. As opposed to empiric treatment of pediatric acute bacterial conjunctivitis in an outpatient setting, in an inpatient setting where causative organisms may be different and antibiotic resistance may be higher, conjunctival swabbing for culture and susceptibilities may be warranted.
Baseline Donor Characteristics in the Cornea Donor Study

Cornea Donor Study Group

Purpose: The Cornea Donor Study (CDS) is an ongoing study that is being conducted to determine whether donor age is related to long-term corneal graft survival. Characteristics of the donor population have been evaluated with respect to donor age, endothelial cell density, and death to preservation interval.

Methods: Within the context of a prospective, double-masked, controlled trial, 1101 donor corneas were assigned without regard to donor age.

Results: Slit-lamp characteristics of the donor corneas showed little variation with donor age, except for the presence of corneal arcus. As death to preservation time decreased, fewer epithelial abnormalities and a lower frequency of stromal edema and Descemet folds were observed. There was little change in the mean of the endothelial cell density with donor age beyond age 60, despite variation.

Conclusion: With respect to donor age, there was little difference in either the slit-lamp characteristics or endothelial cell density of the donor corneas. Fewer epithelial abnormalities were observed with shorter death to preservation time.
Exogenous Gene Delivery and Expression in Human Corneal Stroma Cells Using an Adenoviral Vector

G Amescua, S Pfahler, EC Carlson, Victor L Perez

Purpose: To develop a minimally invasive and efficient means of delivering and expressing a gene of interest in the human corneal stroma.

Methods: Utilizing the technique of intrastromal injection using 1cc syringe and a 30G needle, 5 x 10⁹ pfu of adenoviral vector expressing an EGFP reporter gene driven by a CMV promoter in a volume of 50 µl was delivered to the corneal stroma of human corneal buttons from the Cleveland Eye Bank. The corneal buttons were then cultured in an incubator at 37°C and 5% CO₂ in RPMI media for a total of 14 days. Fluorescent stereo-micrograph was taken every 24 hrs, using an appropriate filter for EGFP. At 7 days corneal buttons were removed from cultures, DAPI stained and confocal microscopy was performed to generate 3D reconstructions from z-stack images in 1 micron increments.

Results: No positive EGFP expression could be detected by fluorescent stereo-microscopy at 24 hrs post-injection; however, expression of the EGFP reporter gene was initially observed at 48 hrs post-injection in 50-60 percent of the total corneal area. The area of expression began to diminish at day 9. By day 14, the corneal area with positive EGFP expression was 19.6 percent. Three-dimensional confocal analysis demonstrated the presence of EGFP was exclusively localized to the stroma.

Conclusions: Ex vivo transfection of human corneal buttons can be achieved with intrastromal injection of adenoviral vector. This ability of intrastromal injection to deliver an adenoviral construct may prove useful as a tool to deliver exogenous genes of interest to the corneal stroma.
Gene Therapy in the Cornea

RR Mohan, A Sharma, Marcelo V Netto, S Sinha, Steven E Wilson

Technological advances in the field of gene therapy has prompted more than 300 phase I and phase II gene-based clinical trials for the treatment of cancer, AIDS, macular degeneration, cardiovascular, and other monogenic diseases. Besides treating diseases, gene transfer technology has been utilized for the development of preventive and therapeutic vaccines for malaria, tuberculosis, hepatitis A, B and C viruses, AIDS, and influenza. The potential therapeutic applications of gene transfer technology are enormous. The cornea is an excellent candidate for gene therapy because of its accessibility and immune-privileged nature. In the last two decades, various viral vectors, such as adeno, adeno-associated, retro, lenti, and herpes simplex, as well as non-viral methods, were examined for introducing DNA into corneal cells in vitro, in vivo and ex vivo. Most of these studies used fluorescent or non-fluorescent marker genes to track the level and duration of transgene expression in corneal cells. However, limited studies were directed to evaluate prospects of gene-based interventions for corneal diseases or disorders such as allograft rejection, laser-induced post-operative haze, herpes simplex keratitis, and wound healing in animal models. We will review the successes and obstacles impeding gene therapy approaches used for delivering genes into the cornea.
Immunohistochemical Detection and Western Blot Analysis of Nitrated Protein in Stored Human Corneal Epithelium

Bennie H Jeng, Karen G Shadrach, David M Meisler, Joe G Hollyfield, Jason T Connor, Thomas Koeck, Kulwant S Aulak, Dennis J Stuehr

While the production of nitric oxide by human corneas in storage has recently been demonstrated, protein nitration as a result of this production has not been demonstrated. In this study, nitrated protein accumulation in the epithelium of stored human corneas was assessed. One half of five donor corneas maintained in storage media for 3 days were prepared for immunohistochemical studies. The other halves remained in storage media for seven additional days and were also processed for immunohistochemistry. Mouse monoclonal antibody to nitrotyrosine adducts was used to define the localization of these epitopes. The density of antibody staining was observed and quantified on a digital camera system and statistically analyzed. Immunostaining in the epithelium was greater in tissues recovered after 10 days in storage compared to the intensity of staining after 3 days of storage (p<0.0001). No staining was evident in the epithelium in sections exposed to non-immune mouse IgG.

Western blot analysis was performed on epithelial cells scraped from corneal surfaces of one-half of 4 donor corneas in storage for 3 days and from the other half at 10 days of storage. Nitrated BSA was used as a positive control. After extraction and homogenization, identical protein concentrations of each sample were loaded per lane on 10% gels and subjected to SDS-PAGE. Proteins were blotted and probed with the anti-nitrotyrosine antibody. Western blot immunoreactivity was detected in epithelial samples at the 3 and 10 day recovery times with the latter samples showing greater staining intensity.

Nitrated protein, thought to indicate toxic peroxynitrite formation, accumulates in the human corneal epithelium with time of storage. Our study shows that there is an association between increased nitrated protein and storage time.
Purpose: We have previously demonstrated that nitric oxide (NO) production occurs in corneal tissue during storage prior to transplantation and that this production, which leads to protein nitration, can be inhibited with the addition of a nitric oxide synthase inhibitor to the corneal storage media. The aim of this study was to determine if the inhibition of NO production would lead to a decrease in immunohistochemical staining for nitrated protein in corneal tissue during storage.

Methods: Paired human corneas stored in Optisol-GS corneal storage media were obtained from the Cleveland Eye Bank. NГ-monomethylene-L-arginine (LMMA), a non-selective nitric oxide synthase inhibitor, was added to 20 ml of storage media of one cornea from each pair of corneas to a final concentration of 2mM. Paired corneas were stored for 3 days and then bisected. One half of each cornea was placed in formalin fixative, and the remaining halves of each cornea were returned to their original storage media and stored for 4 additional days. The remaining tissue was then subjected to the same treatment as the other halves. Mouse polyclonal antibody to nitrotyrosine was used as the primary antibody, and goat anti-mouse biotin-labeled antibody was used as a secondary antibody. The slides were then treated with a peroxidase ABC kit and photographed with a digital camera system.

Results: Less immunohistochemical staining for nitrated protein was observed in epithelial cells which were exposed to LMMA than in those of the control group at day 7 of storage. No substantial differences in immunohistochemical staining occurred at day 3 between the inhibited and control groups.

Conclusions: The addition of a nitric oxide synthase inhibitor to corneal storage media seems to decrease the amount of immunohistochemical staining for nitrated protein in human corneal epithelial cells during storage. This may correlate clinically to less damage to corneal cellular elements during storage due to the toxic effects of protein nitration.
The Immunological Milieu of Human High Risk Corneal Transplant Recipients

A Galor, X Yang, David M Meisler, Victor L Perez

**Purpose:** We have previously demonstrated in a murine model of high risk corneal transplantation that the recipient bed stroma contained macrophages, neutrophils and CD4 T cells. The latter were localized to areas of vascularization. The goal of this work is to characterize the immunologic milieu of high risk human corneal recipients.

**Methods:** Six vascularized high risk corneal beds (1–4 clock hours of vascularization) were collected at the time of corneal transplantation, snap frozen in OCT and sectioned. Immunohistochemical stains for human macrophages (CD11b), vessels (CD31) and CD4/CD8 T cells were performed. Digital images were obtained and quantification of inflammatory cells was calculated in areas of non–vascularization and vascularization, and these were compared to normal non–vascularized control.

**Results:** Similar to the murine model, in 6/6 high risk corneal beds, macrophages were present in the corneal stroma. In 5/6, these were not localized to areas of vascularization. All of the corneas also contained CD4 T cells, and interestingly, CD8 T cells as well. T cells mostly co–localized to areas with blood vessels. Normal control had no inflammatory cells.

**Conclusions:** Similar to the murine high risk cornea model, human high risk vascularized corneal beds are “immunological disprivileged” as macrophages and T cells form a predominant population of inflammatory cells. T cells appear to utilize vascular channels for migration in contrast to macrophages, which do not seem as dependant on blood vessels. In contrast to the murine model, CD8 T cells are also present and seem to co–localize to vascular areas.
“No-Stitch” Triple Procedure

Steven B Koenig, David M Meisler

**Purpose:** To demonstrate the combined use of posterior lamellar endothelial keratoplasty (PLEK) and phacoemulsification (phaco) to perform a “no-stitch” triple procedure.

**Methods:** Phaco and PLEK were performed in a patient with Fuchs dystrophy and cataract. The corneal incisions were self-sealing. The intended postoperative refraction was plano.

**Results:** The refraction at one week postop was +1.00 +0.50 x 105 = 20/30 -1. The graft was clear; the corneal thickness = 737 µ. K readings = 44.12 x 44.62 x 180.

**Conclusion:** Combined phaco and PLEK can produce rapid visual rehabilitation, low postoperative astigmatism, and a predictable postoperative refraction.
Regulation of Neutrophil Migration in the Corneal Stroma by Chemokines and Proteoglycans in Endotoxin-induced Keratitis

EC Carlson, X Yang, C Liu, G Amescua, Victor L Perez

Purpose: How the corneal stromal environments change with respect to chemokine and proteoglycan expression during keratitis is currently unknown. The goal of this study is to determine the role of MIP-2 in inflammatory cell migration through the cornea in lipopolysaccharide-induced keratitis and the impact on keratocan expression.

Methods: Keratitis was induced by intrastromal injection of 2 µg of lipopolysaccharide into the corneal stroma of mice. In order to determine the function of a chemokine, MIP-2, an in vivo migration assay was performed using EGFP-chimeric mice. A scratch was made in the temporal paracentral cornea at time -24 hrs to recruit inflammatory cells to a localized site. At time 0 hrs, fluorescent-labeled LPS was injected into the nasal paracentral cornea and a MIP-2 neutralizing antibody or an IgG control was injected into the central cornea. In vivo fluorescent stereomicrographs were captured and quantitated at various time points to track the response of EGFP-positive inflammatory cells to the LPS stimulus in the presence or absence of MIP-2 neutralizing antibody. Corneas and enucleated eyes were harvested at 6, 24, 48 and 72 hrs following intrastromal injection of LPS and a keratocan western blot was performed. Inflammatory cells were quantitated by performing NIMP-R14 and F4-80 immunohistochemistry on enucleated eye frozen sections.

Results: EGFP-positive bone marrow-derived inflammatory cells migrate into the corneal stroma in response to LPS. MIP-2 neutralization blocked 47 % of inflammatory cell migration through the corneal stroma when compared to an IgG control in response to LPS. Keratocan expression decreased 75% in 24 hrs following intrastromal injection of LPS, but increased 300% between 24 and 48 hrs reaching naïve corneal levels by 72 hrs. The number of neutrophils in the corneal stroma peaked at 24 hrs and began to subside 48 hrs following LPS intrastromal injection correlating with keratocan expression.

Conclusions: Migration of neutrophils through the corneal stroma in LPS-induced keratitis is partially mediated by the chemokine MIP-2. The influx of neutrophils into the corneal stroma results in decreased keratocan levels, which may facilitate neutrophil migration by compromising the integrity of the highly organized stromal matrix.
Role of Early Chemokine Production and Inflammatory Cell Recruitment in High Risk Corneal Transplants

Victor L Perez, JP Rodriguez-Perez, EC Carlson, F Collins, and X Yang

Purpose: To characterize the role of early chemokine production and inflammatory cell recruitment into the cornea following syngeneic and allogeneic high risk corneal transplantation.

Methods: Orthotopic syngeneic (C57BL/6 to C57BL/6) and allogeneic (Balb/c to C57BL/6) corneal grafts were performed in high risk vascularized recipients. Corneal extracts from corneal buttons harvested at 6, 24, 48, 72 hrs and 5, 7 and 14 days after transplantation were prepared and the production of 21 chemokines was assessed using Luminex technology. The recruitment of neutrophils, macrophages and T cells into the host corneal bed and corneal transplant by selective chemokines was assessed and quantified by immunohistochemical staining.

Results: In both syngeneic and allogeneic high risk corneal transplants, there is an influx of neutrophils, macrophages and T cells early on after transplantation. The recruitment of these populations of cells correlated with the production of CXC and CC chemokines such as KC, MIP1, etc. However, IP-10, a CXC chemokine responsible for the recruitment of activated T cells, was produced at higher levels during the first 3 days after transplantation in high risk allogeneic grafts. These correlated with the recruitment and persistence of T cells in the host bed.

Conclusions: Early recruitment of innate inflammatory cells after transplantation in high recipients is similar in syngeneic and allogeneic grafts in response to chemokine production associated with surgical trauma. However, IP-10 production is only produced in allogeneic grafts and these could represent the chemoattractant signal responsible for the recruitment of activated alloantigenic T cells responsible for graft rejection.
Superficial Keratectomy for the Treatment of Epithelial Basement Membrane Abnormalities in Fuchs Endothelial Dystrophy

Bennie H Jeng, David M Meisler

**Purpose:** Epithelial basement membrane abnormalities in eyes with Fuchs endothelial dystrophy can account for vision loss, but it is infrequently targeted for treatment.

**Methods:** Six eyes of four patients with both conditions underwent treatment directed at the epithelial disease.

**Results:** In four eyes of three patients, superficial keratectomy was performed, yielding a mean of 2.25 lines of visual improvement (range: 0–4). One patient elected conservative treatment with hypertonic saline eyedrops for both eyes and gained two lines of vision in each eye.

**Conclusion:** Treatment of epithelial basement membrane changes in eyes with Fuchs endothelial dystrophy can improve visual acuity.
Thin-Strip Conjunctival Autograft in Pterygium Surgery

William J Dupps Jr, Bennie H Jeng, David M Meisler

**Purpose:** To determine the efficacy of thin-strip conjunctival autograft in preventing the recurrence of pterygium.

**Methods:** Retrospective review of 21 consecutive surgeries for 17 primary and 4 recurrent pterygia employing a thin-strip conjunctival autograft technique. In all cases, a 2mm wide autograft was secured at the limbus, leaving 2-3 mm of bare sclera between the graft and the conjunctival margin. The latter was secured with sclera sutures.

**Results:** Over a mean follow-up of 30 months (median: 17; range 1-126), only one recurrence occurred.

**Conclusion:** Thin-strip conjunctival autografting appears to be an effective technique for preventing pterygium recurrence.
Use of Ultrasound Biomicroscopy in the Evaluation for Repair of Descemet’s Membrane Detachments

Bennie H Jeng, David M Meisler

**Purpose:** To describe the utility of ultrasound biomicroscopy (UBM) in aiding the delineation of Descemet’s membrane detachments prior to surgical repair.

**Methods:** Descemet’s membrane detachments after clear corneal cataract surgery in five eyes of five patients were repaired using a combination of intracameral sulfur hexafluoride (SF6) gas and full-thickness 10-0 nylon sutures. In two of these patients, UBM was used preoperatively to aid in the delineation of the extent of the detachments to ensure proper suture placement.

**Results:** UBM was able to demonstrate the full extent of the Descemet’s membrane detachments in both patients in which this technique was used. In all patients, successful reattachment was achieved. Because of irreversible corneal edema, one patient required penetrating keratoplasty to restore functional vision.

**Conclusions:** UBM is a useful tool to aid in delineating the full extent of Descemet’s membrane detachments, especially in eyes where diffuse corneal edema precludes an adequate view of the posterior cornea and anterior chamber.
Section 2

Glaucoma
Cochlin and Glaucoma: A Mini-Review

Sanjoy K Bhattacharya, Neal S Peachey, John W Crabb

Primary open angle glaucoma (POAG) is a leading cause of late onset, progressive, irreversible blindness and, although its etiology is poorly understood, elevated intraocular pressure (IOP) often appears to be a contributory factor. Proteomic and Western analyses of trabecular meshwork (TM) from patients with POAG and age-matched controls originally implicated cochlin as possibly contributing to glaucoma pathogenesis. Cochlin deposits were subsequently detected in glaucomatous but not in control TM and older glaucomatous TM was found to contain higher levels of cochlin and significantly lower amounts of collagen type II. More recently, similar results were reported in DBA/2J mice, which at older ages develop elevated IOP, retinal ganglion cell degeneration and optic nerve damage. Notably, cochlin was absent in TM from C57BL/6J, CD1 and BALBc/ByJ mice, which do not exhibit elevated IOP or glaucoma. Cochlin was found in the TM of very young DBA/2J mice, prior to elevated IOP, suggesting that over time the protein may contribute to the events leading to increased IOP and optic nerve damage. Here we review these findings and describe how future studies in DBA/2J mice can help resolve whether cochlin plays a causal role in mechanisms of POAG and elevated IOP.
Cochlin Deposits in the Trabecular Meshwork of the Glaucomatous DBA/2J Mouse

Sanjoy K Bhattacharya, SP Annangudi, RG Salomon, Rachel W Kuchtey, Neal S Peachey, John W Crabb

Cochlin deposits were observed in the trabecular meshwork (TM) of 8-month-old glaucomatous DBA/2J mice, coincident with the reported onset of increased intraocular pressure and optic nerve damage. An age-dependent increase in cochlin was observed up to 10 months of age and was paralleled by a decrease in type II collagen. Similar expression patterns exist in the TM of humans with primary open-angle glaucoma. Cochlin deposits, absent in non-glaucomatous mouse and human TM, may disrupt the TM extracellular matrix and obstruct aqueous humor circulation. Studies of DBA/2J mice offer promise for understanding the role cochlin may play in glaucoma.
Comparison of Optical Coherence Tomography and Ultrasound Biomicroscopy for Detection of Narrow Anterior Chamber Angles

S Radhakrishnan, J Goldsmith, David Huang, V Westphal, DK Dueker, AM Rollins, JA Izatt, Scott D Smith

Objective: To assess the accuracy of classification of narrow anterior chamber (AC) angles using quantitative imaging by optical coherence tomography (OCT) and ultrasound biomicroscopy (UBM).

Design: Observational comparative study.

Methods: A high-speed (4000 axial scans/s) anterior segment OCT prototype was developed using a 1.3-micron light source. Seventeen normal subjects (17 eyes) and 7 subjects (14 eyes) with angle closure glaucoma were enrolled. All subjects underwent gonioscopy, OCT, and UBM. Quantitative AC angle parameters (angle opening distance, angle recess area, and the trabecular-iris space area [a new parameter we have defined]) were measured from OCT and UBM images using proprietary processing software.

Main outcome measures: Specificity and sensitivity in identifying narrow angles with image-derived AC angle parameters.

Results: Eight of 31 eyes were classified as having narrow angles (Shaffer grade < or =1 in all quadrants). The AC angle parameters measured by both OCT and UBM had similar mean values, reproducibility, and sensitivity-specificity profiles. Both OCT and UBM showed excellent performance in identifying eyes with narrow angles. Areas under the receiver operating characteristic curves for these parameters were all in the range of 0.96 to 0.98.

Conclusions: Optical coherence tomography was similar to UBM in quantitative AC angle measurement and detection of narrow angles. In addition, it was easier to use and did not require contact with the eye. Optical coherence tomography is a promising method for screening individuals at risk for angle closure glaucoma.
Comparison of Trabeculectomy with Mitomycin C and Ahmed Glaucoma Valve Implantation for the Treatment of Uveitic Glaucoma

Rachel W Kuchtey, Careen Y Lowder, Scott D Smith

Purpose: To compare the safety and efficacy of trabeculectomy with mitomycin-C (MMC) and Ahmed Glaucoma Valve implantation in the treatment of uveitic glaucoma.

Methods: We performed a retrospective chart review of patients with uveitic glaucoma who underwent trabeculectomy with mitomycin C or Ahmed glaucoma implant during a four-year period. Patients were identified from a computerized database of patients who underwent trabeculectomy with MMC or implantation of an Ahmed valve and had been treated for anterior or posterior uveitis of any etiology in our Uveitis Service. Charts were abstracted to identify the patients’ demographic and clinical characteristics. Failure of glaucoma surgery was defined by an intraocular pressure (IOP) > 21 mm Hg with glaucoma medications, loss of light perception, or the need for re-operation to control the IOP.

Results: A total of 38 eyes of 27 patients with at least 12 months follow-up were identified. The average length of follow-up was 28.7 months. Twenty-six eyes underwent trabeculectomy with MMC, and 12 eyes underwent Ahmed Valve implantation. There were no statistically significant differences in age, sex, race, baseline IOP or number of glaucoma medications between the two groups. No significant differences in mean IOP were observed between the two groups at any point during follow-up. The cumulative success rates for trabeculectomy and Ahmed valve implantation were 77% and 100% respectively, a difference that was not statistically significant (p>0.1). The number of glaucoma medications required by patients in the Ahmed implant group was higher than that in trabeculectomy group at 6 months (0.9 for Ahmed group and 0 for trabeculectomy group respectively, p=0.0013). A similar difference in need for glaucoma medications was seen at 12 month follow-up. However, there was no statistically significant difference in the number of glaucoma medications at final follow-up (0.7 for Ahmed group and 0.9 for trabeculectomy group respectively, p>0.5). The rates of hypotony and other complications did not differ between the two groups.

Conclusions: At medium-term follow-up, Ahmed Valve implantation and trabeculectomy with MMC have comparable efficacy and safety in the management of uveitic glaucoma. However, a greater need for postoperative glaucoma medications is required following placement of an Ahmed implant.
Converting to SITA-Standard from Full-Threshold Visual Field Testing in the Follow-up Phase of a Clinical Trial

David C Musch, Brenda W Gillespie, Bonnie M Motyka, Leslie Niziol, Richard P Mills, Paul R Lichter

**Purpose:** To evaluate the impact of converting from Humphrey 24-2 full-threshold (FT) visual field (VF) testing to SITA-Standard (SS) VF testing during the follow-up phase of a clinical trial.

**Methods:** VF data were obtained from 243 patients in the Collaborative Initial Glaucoma Treatment Study (CIGTS) who had follow-up visits in 2004. FT and SS VF tests were performed in random order on the same day.

**Results:** The average duration of the SS test (6.3 minutes) was shorter ($P < 0.0001$, paired $t$-test) than the FT test (11.8 minutes). The mean deviation did not differ between SS and FT testing. A small difference was found in the pattern SD (PSD) ($P = 0.02$). The mean CIGTS score from the FT test (4.5) was significantly lower ($P < 0.0001$) than the mean CIGTS score from the SS test (6.0). Although the two tests yielded identical Glaucoma Hemifield Test (GHT) results in 179 patients (76%), 16 patients had a normal GHT result on FT testing and an SS test result that was outside normal limits. Six patients had the reverse finding. The most significant factor associated with an increased (positive) difference between the CIGTS VF score generated from SS and FT testing was conducting the FT test first ($P < 0.0001$).

**Conclusions:** Although SS and FT testing yielded very similar mean deviation results, the CIGTS VF score and GHT differed between SS and FT tests. Changing the approach used to measuring a study's primary VF outcome should be accompanied by a critical evaluation of the change’s impact.
Detection of Patients at Risk of Angle-closure Using Anterior Segment OCT

WP Nolan, J See, T Aung, Z Ce, S Radhakrishnan, DS Friedman, Scott D Smith, PT Chew

**Purpose:** To compare a new non-contact imaging method, the anterior segment optical coherence tomograph (AS-OCT), with gonioscopy in the detection of occludable angles.

**Methods:** Patients attending the glaucoma service at the National University Hospital in Singapore were recruited to this preliminary study evaluating a new prototype of the AS-OCT (Carl Zeiss Meditec, Inc, Dublin, CA, USA). All subjects underwent gonioscopy using the Goldmann 2-mirror lens under dim light conditions by a single observer. The angle width was graded according to the Scheie grading system for all four quadrants of the angle in both eyes. A quadrant of the angle was defined as occludable on gonioscopy if trabecular meshwork was not visible without indentation. All subjects underwent imaging in the sitting position with the AS-OCT under dark and light conditions by a separate single observer. An image of the nasal, temporal and inferior quadrants was recorded for each eye, and the quadrant was defined as closed if peripheral iris was apposed to the trabecular meshwork anterior to the scleral spur, and defined as narrow if a small slit was visible between the peripheral iris and the trabecular meshwork/angle anterior to the scleral spur.

**Results:** Preliminary data have been obtained from 54 eyes of 29 patients. In 28 eyes, the temporal quadrant of the angle was defined as occludable by gonioscopy. AS-OCT images demonstrated narrow or closed angles in 24 of these eyes (85.7%). In 46 eyes the inferior angle was defined as occludable by gonioscopy of which the AS-OCT imaging identified 40 (87%) as narrow or closed. Of 19 eyes in which the nasal quadrant was defined as occludable the AS-OCT correctly identified 17 (89.5%). In total the AS-OCT correctly identified 81/93 (87.1%) occludable angle quadrants as narrow or closed.

**Conclusions:** This new prototype of the AS-OCT has the advantage of providing a rapid non-contact method of imaging the drainage angle. These preliminary data demonstrate that the device performs well in identifying patients with angle-closure when compared with gonioscopy.
Effect of Laser Peripheral Iridotomy on Angle Configuration in Eyes with Angle Closure: An Evaluation by the Anterior Segment OCT

J See, PT Chew, WP Nolan, C Zheng, R Sunita, S Smith, DS Friedman, T Aung

**Purpose:** To evaluate the effect of laser peripheral iridotomy on angle configuration in eyes with angle closure, using the anterior segment optical coherence tomograph (AS-OCT).

**Methods:** Patients attending the glaucoma service at the National University Hospital, Singapore were recruited into this study. Those identified to have angle closure underwent imaging with the AS-OCT (Carl Zeiss Meditec, Inc, Dublin, CA, USA) before and after laser iridotomy. Images of the nasal, temporal and inferior quadrants were acquired in all eyes. The AS-OCT images of the right eyes obtained under dark conditions were analysed for angle opening distance at 500µm (AOD$_{500}$), area of recessed angle (ARA$_{500}$) and trabecular-iris space area (TISA).

**Results:** Ten patients were analysed in this preliminary study, including 4 men and 6 women. The mean age was 62.4 years ± 8.3. After laser iridotomy, there was a significant increase in AOD$_{500}$ in the nasal, temporal and inferior angles (p=0.03, p=0.02, p=0.01 respectively). However, ARA$_{500}$ did not show a significant change post-laser iridotomy and TISA showed a significant increase only in the temporal angles.

**Conclusions:** In eyes with angle closure, the angle opening distance (AOD$_{500}$) was found to increase significantly after laser iridotomy. The AS-OCT is a promising imaging device that allows assessment of changes in angle configuration after laser iridotomy in eyes with angle closure.
Glaucoma in Patients with Ocular Inflammatory Disease

Rachel W Kuchtey, Careen Y Lowder, Scott D Smith

Uveitic glaucoma can pose some of the most challenging management problems faced by the ophthalmologist. A better understanding of the pathogenesis of glaucoma associated with ocular inflammatory disease is an important key to making appropriate therapeutic decisions. This article provides an update on recent advances in understanding the epidemiology and pathogenesis of uveitic glaucoma, as well as developments in the diagnosis and management of this condition.
Illumination-Induced Changes in the Angle Configuration: An Evaluation by Anterior Segment Optical Coherence Tomography

S Radhakrishnan, J See, PT Chew, WP Nolan, Z Ce, DS Friedman, T Aung, Scott D Smith

**Purpose:** To evaluate illumination-induced changes in the angle configuration by using anterior segment optical coherence tomography (AS-OCT).

**Methods:** Patients attending the glaucoma service at the National University Hospital, Singapore, were recruited into this study. Subjects included patients with primary angle closure glaucoma, primary open angle glaucoma, anatomically narrow angles and glaucoma suspects. Patients were imaged with the AS-OCT system (Carl Zeiss Meditec, Inc., Dublin, CA, USA) under dark conditions as well as bright illumination. Images of the nasal and temporal quadrants were acquired in all eyes. The AS-OCT images of the right eyes were analyzed for angle opening distance at 500 µm (AOD$_{500}$), angle recess area at 500 µm and 750 µm (ARA$_{500}$ and ARA$_{750}$) and trabecular-iris space area at 500 µm and 750 µm (TISA$_{500}$ and TISA$_{750}$).

**Results:** Twenty-nine patients were analyzed in this preliminary study. The mean age was 62.4 ± 8.3 years. Under dark conditions, the mean value of AOD$_{500}$ was significantly smaller than that measured under bright illumination in both the nasal (132 µm vs. 211 µm, p<0.0001) and temporal (138 µm vs. 240 µm, p<0.0001) quadrants. The mean ARA$_{500}$ was also smaller under dark conditions (nasal quadrant: 0.06mm$^2$ vs. 0.08mm$^2$, p=0.003; temporal quadrant: 0.07mm$^2$ vs. 0.11mm$^2$, p<0.0001). The other angle parameters measured showed a similar decrease under dark conditions. The temporal quadrant showed a trend toward being wider than the nasal quadrant with the difference being significant for ARA$_{500}$ & TISA$_{500}$ (p=0.01) as well as ARA$_{750}$ and TISA$_{750}$ (p=0.03) under bright illumination.

**Conclusions:** Anterior segment optical coherence tomography is a promising imaging device that allows assessment of illumination-induced changes in the angle configuration. Using this instrument we observed a significant decrease in angle width under dark conditions. This finding suggests that the prevalence of anatomically narrow angles may be underestimated if screening protocols do not include evaluation under conditions of dim illumination.
Outcomes of Cataract Surgery in Eyes with a Filtering Bleb

Edward J Rockwood

**Introduction:** To define outcomes of cataract surgery in eyes with a filtering bleb and risk factors for filtering surgery failure.

**Methods:** Prospective, uncontrolled study of 224 eyes with cataract surgery in 199 patients with a functioning glaucoma filtering bleb. Patients received subconjunctival injection of triamcinolone acetonide at the end of surgery, and hourly topical prednisolone 1% postoperatively. Statistical analysis and a Kaplan-Meier survival analysis were performed.

**Results:** Filtering surgery failure occurred in 8/31 (25.8%) ECCE and in 12/193 (6.2%) phacoemulsification procedures. Uveitis, younger age, and the development of central retinal vein occlusion with neovascular glaucoma were additional risk factors for failure. Failures occurred at a mean of 39.7 months after cataract surgery (range: 0.1 to 96 months). Visual acuity improved in 187 (83.5%), remained unchanged in 14 (6.3%), and was worse in 23 (10.3%). Mean IOP increased from 11.3 mm Hg preoperatively to 15.4 mm Hg postoperatively and mean glaucoma medication increased from 0.1 preoperatively to 0.7 postoperatively. Bullous keratopathy developed in 6 (2.7%) eyes and late bleb-related endophthalmitis in 2 (0.9%) eyes. Other reasons for poor postoperative visual acuity included preoperative fixation loss from glaucoma, wet age-related macular degeneration in 2 eyes, diabetic macular edema in 3 eyes, and CRVO with NVG in 2 eyes.

**Discussion:** Cataract surgery increases mean IOP and the need for glaucoma medication. Filtering surgery survival was substantially improved over the Rebollo-da report. Most (83.5%) of eyes experienced improved long-term visual acuity. Other events not related to glaucoma or filtering surgery failure such as DME, wet AMD and CRVO, caused reduced postoperative visual acuity in some patients.

**Conclusions:** Glaucoma filtering surgery failure is more likely in younger patients, uveitis, after ECCE, and after CRVO with NVG. Intensive postoperative corticosteroid use may improve filtering surgery survival.

Outcomes of Glaucoma Implant Combined Surgical Procedures

Edward J Rockwood, David M Meisler, Jonathan E Sears

Introduction: The purpose of this study is to report the outcomes of glaucoma implant surgery performed in combination with other surgical procedures in patients with glaucoma and other disorders including cataract, severe conjunctival scarring, bullous keratopathy, vitreous hemorrhage, and macular pucker.

Methods: A noncomparative, consecutive series of 51 eyes of 51 patients who had glaucoma implant surgery combined with pars plana vitrectomy, penetrating keratoplasty, and/or cataract surgery performed between 5/30/01 and 10/31/05. Survival analysis was performed. Glaucomas included angle closure, uveitic, neovascular, and congenital. Twenty-three patients had previous failed trabeculectomy surgery.

Results: Visual acuity improved in 28, remained the same in 16, and worsened in 7 patients. Worsening of visual acuity occurred because of graft failures, and glaucoma implant failures (two each), and corneal ulcer, glaucoma implant leak, and worsening of macular degeneration (one each). Mean IOP decreased from 29.4 to 16.6 mm Hg and mean number of glaucoma medications was reduced from 2.8 to 0.7. At time of last follow-up, 47 (92%) eyes had successful IOP control, 3 eyes required reoperation (two had a second glaucoma implant) to control IOP, and one eye required glaucoma implant removal for chronic leak. Five of 7 corneal transplants performed in conjunction with glaucoma implant have survived. Despite pars plana vitrectomy, one intravitreal tube became obstructed with vitreous.

Discussion: Best outcomes occurred in patients with combined glaucoma implant and cataract surgery and in eyes with glaucoma implant surgery combined with pars plana vitrectomy and membrane peel. Corneal graft survival may be improved because of insertion of the tube into the vitreous rather than the anterior chamber.

Conclusions: Glaucoma implant surgery can be successfully performed in combination with cataract, corneal transplant, and vitrectomy surgery in high risk eyes with multiple ocular disorders. Some eyes require a second glaucoma implant and most corneal grafts survive.
Oxidative Protein Modifications in Glaucomatous Trabecular Meshwork

SP Annangudi, Edward J Rockwood, Scott D Smith, RG Salomon, Sanjoy K Bhattacharya, John W Crabb

**Purpose:** To determine whether oxidative protein modifications are elevated in the glaucomatous trabecular meshwork and possibly play a role in glaucoma pathogenesis.

**Methods:** Trabecular meshwork (TM) was dissected from normal human cadaver eyes from the Cleveland Eye Bank and from primary open angle glaucoma (POAG) patients undergoing trabeculectomy at the Cole Eye Institute. Protein was extracted by homogenization in 100 mM Tris-Cl buffer pH 7.8 containing 5 mM dithiotheritol, 1 mM SnCl₂, 50 mM NaHPO₄, 1 mM diethylenetriamine-pentaacetic acid, 100 mM butylated hydroxy toluene and 0.5% SDS. Western analyses for oxidative protein modification were performed with antibodies to carboxyethyl-pyrrole (CEP), hydroxynonenal (HNE), isolevuglandin (Iso[4]LGE₂) and argpyrimidine (AGEs).

**Results:** HNE immunoreactivity was found in 9/12 POAG TM but in no normal TM (0/12). AGE immunoreactivity was found in 3/4 POAG TM and no normal TM (0/4). Iso[4]LGE₂ immunoreactivity was found in 8/8 POAG TM and in 2/8 normal TM. CEP immunoreactivity was not observed in either POAG or normal TM.

**Conclusions:** Protein modifications generated from the oxidation of lipids and sugar appear to be more prevalent in glaucomatous TM than in normal TM. Such oxidative modifications may contribute to blockage of aqueous humor circulation and increased intraocular pressure in POAG.
Perioperative Complications of Trabeculectomy in the Collaborative Initial Glaucoma Treatment Study (CIGTS)

HD Jampel, David C Musch, Brenda W Gillespie, Paul R Lichter, MM Wright, KE Guire, the CIGTS Study Group

Purpose: To describe the incidence of, and risk factors for, surgical complications reported during and within the first post-operative month after trabeculectomy in the Collaborative Initial Glaucoma Treatment Study (CIGTS).

Design: Review of prospectively collected data from a multicenter, randomized clinical trial.

Methods: Complications were tabulated for the 300 CIGTS patients randomized to surgery. Logistic regression analyses were used to identify risk factors for complications.

Results: Among the 300 patients randomized to initial surgery, 465 trabeculectomies were performed. Intraoperative complications were reported in 55 eyes (12%). The most frequent reported complications were anterior chamber bleeding during surgery (37 eyes, 8%) and conjunctival buttonhole (five eyes, 1%). Early post-operative complications were reported in 232 eyes (50%). Complications with a frequency over 10% included shallow or flat anterior chamber (62 eyes, 13%), encapsulated bleb (56 eyes, 12%), ptosis (55 eyes, 12%), serous choroidal detachment (52 eyes, 11%), and anterior chamber bleeding or hyphema (48 eyes, 10%). There were three localized suprachoroidal hemorrhages (0.7%) and no cases of endophthalmitis. Older patients were more likely to experience serous choroidal detachment, new anterior or posterior synechiae, and wound leak. Blacks were less likely to experience anterior chamber bleeding, but more likely to experience post-operative ptosis. The number of subjects experiencing bilateral complications was higher than that which would have been predicted by chance alone.

Conclusions: The incidence of transient and self-limiting complications was high in the perioperative period, but we observed few complications with the potential to cause severe sustained vision loss in this group of previously untreated eyes.
Proteomics Reveal Cochlin Deposits Associated with Glaucomatous Trabecular Meshwork

Sanjoy K Bhattacharya, Edward J Rockwood, Scott D Smith, Vera L Bonilha, John S Crabb, Rachel W Kuchtey, Nahid G Robertson, Neal S Peachey, Cynthia C Morton, John W Crabb

The etiology of primary open angle glaucoma, a leading cause of age-related blindness, remains poorly defined, although elevated intraocular pressure (IOP) contributes to the disease progression. To better understand the mechanisms causing elevated IOP from aqueous humor circulation, we pursued proteomic analyses of trabecular meshwork (TM) from glaucoma and age-matched control donors. These analyses demonstrated that Cochlin, a protein associated with deafness disorder DFNA9, is present in glaucomatous but absent in normal TM. Cochlin was also detected in TM from the glaucomatous DBA/2J mouse preceding elevated IOP but found to be absent in three other mouse lines that do not develop elevated IOP. Histochemical analyses revealed co-deposits of Cochlin and mucopolysaccharide in human TM around Schlemm's canal, similar to that observed in the cochlea in DFNA9 deafness. Purified Cochlin was found to aggregate after shear stress and to induce the aggregation of TM cells in vitro. Age-dependent in vivo increases in Cochlin were observed in glaucomatous TM, concomitant with a decrease in type II collagen, suggesting that Cochlin may disrupt the TM architecture and render components like collagen more susceptible to degradation and collapse. Overall, these observations suggest that Cochlin contributes to elevated IOP in primary open angle glaucoma through altered interactions within the TM extracellular matrix, resulting in cell aggregation, mucopolysaccharide deposition, and significant obstruction of the aqueous humor circulation.
Proteomics Suggests Glaucoma Pathogenesis May Involve Optic Nerve Peptidylarginine Deiminase

Sanjoy K Bhattacharya, John S Crabb, Xiarong Gu, John W Crabb

Purpose: To identify optic nerve proteins and protein modifications associated with the pathology of primary open angle glaucoma (POAG).

Methods: Normal human and POAG donor eyes were obtained from NDRI in compliance with the declaration of Helsinki. Optic nerve was dissected and protein was extracted in 7M urea and 2M thiourea containing 3% dodecylmaltoside. Following 1D SDS-PAGE, gel bands were excised, digested in-situ with trypsin and proteins identified by capillary LC MS/MS. Western analyses were performed with antibodies to citrulline (Upstate Biotechnology), to protein arginine methylation (Abcam), to peptidylarginine deiminase type II (PAD2) and to myelin basic protein (MBP).

Results: Proteomic analyses of optic nerve from healthy (n=12) and POAG (n=12) donors detected PAD2 uniquely in POAG tissue. Western analysis supported elevated levels of PAD2 in POAG compared to normal optic nerve. Western analyses also revealed increased levels of citrullination, including citrullinated MBP, and decreased protein methylation in POAG optic nerve.

Conclusions: PAD2 under [Ca^{2+}] modulation converts protein arginine to citrulline. Others have associated this enzyme with rat brain neurodegeneration. We observed increased PAD2 protein and PAD2 activity in POAG optic nerve. This may be a consequence of intracellular [Ca^{2+}] imbalance. Furthermore, citrullination and decreased protein methylation may disrupt myelination and contribute to optic nerve degeneration. We hypothesize that deiminase activity may be associated with glaucoma pathogenesis.
Trabeculectomy as Initial Treatment for OAG Patients with Substantial VF Defects

Paul R Lichter, David C Musch, Brenda W Gillespie, Leslie Niziol, the CIGTS Study Group

Introduction: Traditional therapy for newly diagnosed open-angle glaucoma is medication. Whether initial surgery should be preferred might depend on the severity of visual field (VF) loss at baseline.

Methods: In the Collaborative Initial Glaucoma Treatment Study (CIGTS), 607 patients with newly diagnosed open-angle glaucoma were randomized to initial treatment with medication or with trabeculectomy and underwent regular follow-up. Factors associated with mean deviation (MD) over time were assessed on data collected through 7.5 years of follow-up. Also, a predictive model was used to evaluate the association of IOP history during the first three years of treatment on subsequent VF progression.

Results: There was a highly significant (P<0.0001) association of baseline MD with subsequent MD and a very significant (P<0.0003) interaction of baseline MD severity with treatment. For patients with a mild defect in baseline MD (MD -2dB), initial treatment with medicines or with trabeculectomy resulted in similar MD values over time. But for patients with an MD of -10dB or worse at baseline, those randomized to medicine showed increasingly worse MDs over time compared to those randomized to trabeculectomy. The more advanced VF loss surgical group had significantly lower mean, maximum and standard deviation of IOP as compared to the medically treated group.

Discussion: While interim CIGTS results showed that initial treatment with medications and initial treatment with surgery resulted in similar VF outcomes in the overall study population, when we stratified the groups by severity of VF loss at baseline and followed the patients for a longer period of time, surgically treated patients with more severe baseline VF loss showed better MD values than medically treated patients. This effect is associated with IOP differences in the two groups.

Conclusions: Filtering surgery may be the best initial treatment for patients with open-angle glaucoma who have substantial VF loss at diagnosis.
Section 3

Oculoplastics
Ptosis and Orbital Fat Prolapse after Posterior Sub-Tenon’s Capsule Triamcinolone Injection

AJ Dal Canto, Julian D Perry

**Purpose:** To describe the occurrence of orbital fat prolapse and blepharoptosis after posterior sub-Tenon (PST) triamcinolone injection.

**Design:** Retrospective review of consecutive case series.

**Participants:** Patients with ptosis and orbital fat herniation after PST triamcinolone injection.

**Methods:** Charts of all patients with ptosis and orbital fat herniation presenting after PST triamcinolone injection to the oculoplastics service of the Cole Eye Institute between 1999 and 2003 were reviewed. Charts were reviewed for patient age, indication, dates of injections, time to patient complaint or time to referral for ptosis, and marginal reflex distance (MRD1).

**Main Outcome Measures:** Ptosis and orbital fat herniation after PST triamcinolone injection.

**Results:** Eleven patients with a history of ipsilateral PST triamcinolone injections were seen with ptosis and orbital fat prolapse. Ten charts were available for review. Mean patient age was 64 years (range, 45-78 years). Patients underwent 1 to 9 ipsilateral injections, and 2 patients underwent bilateral injections. Patients were seen for ptosis evaluation on average 22.5 months (range, 3-56 months) after the initial injection, and 6.6 months (range, 0-20 months) after the most recent injection. All patients demonstrated significant orbital fat prolapse in conjunction with statistically significant ptosis (P = 0.016). Tissue was obtained in 3 cases. Histologic findings in 1 case showed orbital fat infiltrated by histiocytes that seemed to contain phagocytosed material.

**Conclusions:** Posterior sub-Tenon triamcinolone injection may cause ptosis associated with orbital fat prolapse. This finding may be a relatively common complication of PST triamcinolone injection. We recommend counseling patients about this risk before PST triamcinolone injection.
Modifying Brow Position with Botulinum Toxin

JA Foster, PL Proffer, LH Proffer, AE Wulc, Julian D Perry

Objective: To discuss historical information, anatomic considerations, patient selection and injection techniques for modifying brow position with botulinum toxin.

Methods: The history of medical botulinum toxin use is discussed, and its neuromuscular effects described. The facial musculature underlying brow position is reviewed. Patient evaluation techniques are addressed. Injection techniques to alleviate glabellar furrows and raise the eyebrows are discussed.

Results: Botulinum toxin type A has been used successfully to address glabellar rhytides and modify brow position. The substance can be used to weaken all four brow depressors: the corrugator supracilii, depressor supercilii, procerus and orbicularis oculi muscles.

Conclusion: Botulinum toxin type A can be used to successfully modify brow position in selected patients.
Section 4
Ophthalmic Anesthesia
Adverse Event Reporting: Lessons Learned from 4 years of Florida Office Data

B Coldiron, AH Fisher, E Adelman, CB Yelverton, R Balkrishnan, Marc A Feldman, SR Feldman

**Background:** Patient safety regulations and medical error reporting systems have been at the forefront of current health care legislature. In 2000, Florida mandated that all physicians report, to a central collecting agency, all adverse events occurring in an office setting.

**Purpose:** To analyze the scope and incidence of adverse events and deaths resulting from office surgical procedures in Florida from 2000 to 2004.

**Methods:** We reviewed all reported adverse incidents (the death of a patient, serious injury and subsequent hospital transfer) occurring in an office setting from March 1, 2000, through March 1, 2004, from the Florida Agency for Health Care Administration. We determined physician board certification status, hospital privileges and office accreditation via telephone follow-up and Internet searches.

**Results:** Of 286 reported office adverse events, 77 occurred in association with an office surgical procedure (19 deaths and 58 hospital transfers). There were seven complications and five deaths associated with the use of intravenous sedation or general anesthesia. There were no adverse events associated with the use of dilute local (tumescent) anesthesia. Liposuction and/or abdominoplasty under general anesthesia or intravenous sedation were the most common surgical procedures associated with a death or complication. Fifty-three percent of offices reporting an adverse incident were accredited by the Joint Commission on Accreditation of Healthcare Organizations, American Association for Accreditation of Ambulatory Surgical Facilities, or American Association for Ambulatory Health Care. Ninety-four percent of the involved physicians were board certified, and 97% had hospital privileges. Forty-two percent of the reported deaths were delayed by several hours to weeks after uneventful discharge or after hospital transfer.

**Conclusions:** Requiring physician board certification, physician hospital privileges or office accreditation is not likely to reduce office adverse events. Restrictions on dilute local (tumescent) anesthesia for liposuction would not reduce adverse events and could increase adverse events if patients are shifted to riskier approaches. State and/or national legislation establishing adverse event reporting systems should be supported and should require the reporting of delayed deaths.
Section 5

Ophthalmic Genetics
A Gene for X-Linked Retinitis Pigmentosa Maps to Xq28 in a Large Ohio Family

A Melamud, G-Q Shen, L Li, D Chung, E Simpson, Stephanie A Hagstrom, Q Wang, Elias I Traboulsi

**Purpose**: To map the gene for an X-linked form of retinitis pigmentosa.

**Methods**: We examined 22 members of a family in which retinitis pigmentosa appeared to be transmitted in an X-linked fashion. Complete ocular examinations were performed and fundus photographs and electroretinograms were obtained. Mutation analysis of the RPGR and RP2 genes were done using published standard methods. Affected and non-affected family members were genotyped for 18 polymorphic markers (ABI Biosystems Inc., Foster City, CA) on the X-chromosome spaced at 10cM intervals. PCR was performed using the ABI PCR 9700 system. Samples were run on the ABI 3100 genetic analyzer. The results were analyzed with the GeneMapper 1.1 software.

**Results**: Visual acuity in the five affected individuals ranged from 20/40 to 20/75. All five described noticing the onset of night blindness and color vision defects symptoms in the second decade of life, with the earliest at age 13. All affected individuals failed the Ishihara color plate test. All had evidence of constricted peripheral vision by Goldman visual field testing. Indirect ophthalmoscopy revealed peripheral bone spicules, waxy pallor of the optic nerve, and attenuated retinal blood vessels. Haplotype analysis revealed segregation of the disease phenotype with markers at Xq28. A LOD score of 2.17 was obtained with marker DXS1073.

**Conclusions**: We present evidence for a possible sixth XLRP locus at Xq28. The clinical phenotype is not significantly different from other types of X-linked RP.
An Arg311Gln NR2E3 Mutation in a Family with Classic Goldmann-Favre Syndrome

SH Chavala, A Sari, Hilel Lewis, GJ Pauer, E Simpson, Stephanie A Hagstrom, Elias I Traboulsi

Goldmann-Favre syndrome (GFS) is one of the rarest inherited vitreoretinal dystrophies that manifests with hemeralopia, degenerative vitreous changes, peripheral and central retinoschisis, a liquefied vitreous cavity with preretinal band-shaped structures, macular oedema, cataract formation, and an abnormal electroretinogram (ERG). The term “clumped pigmentary retinal degeneration” (CPRD) describes a group of patients with decreased night and peripheral vision who have round and irregular clumps of pigment in the mid-peripheral fundus with little or no evidence of bone spicule formation. This pattern of pigmentation occurs in retinitis pigmentosa (RP) with preserved para-arteriolar retinal pigment epithelium (PPRPE), enhanced S-cone syndrome (ESCS), and GFS, and these disorders share common mutations in the NR2E3 gene, which is involved in retinal cell fate determination.

We present clinical and molecular genetic studies of a family from the United Arab Emirates with a classic GFS phenotype and a mutation in the NR2E3 gene.
Expression of PAX6 and BetaIII Tubulin in Fetal Eyes of Ski-Deficient Mice with PHPV and Peters Anomaly

P McGannon, Y Miyazaki, P Gupta, Elias I Traboulsi, C Colmenares

Purpose: Persistent hyperplastic primary vitreous (PHPV) is an uncommon developmental ocular malformation often associated with additional anterior segment and retinal abnormalities. The eyes of mice lacking the Ski proto-oncogene display several of the characteristics of PHPV in addition to what resembles Peter’s anomaly. We review the spectrum of ocular malformations in fetal Ski-/- mice and we report on the expression of Pax6 and B= tubulin in these eyes.

Methods: Morphologic and histologic analyses of Ski-/- mice were used to document ocular abnormalities and compared to eye morphology of normal, +/- and +/+ littermates. Immunohistochemical studies were used to examine the expression of Pax6 as a marker of eye development, and of B=tubulin as a neural cell marker to determine the cellular origin of abnormal vitreal tissue. Assays for apoptosis were used to test the hypothesis that alterations in programmed cell death underlie the observed abnormalities. Mouse eyes were harvested between E16 and E18.

Results: The incidence of PHPV and microphthalmia in Ski-deficient fetuses was 100%. Other abnormalities included some degree of anterior segment and lens dysgenesis in all animals, retinal folds (78%), chorioretinal coloboma (78%), and Peter’s anomaly (44%). The severity of these phenotypes was variable even in a highly homogeneous genetic background. PHPV was characterized by variable amounts of retrodental fibrous tissue. This mesenchymal tissue did not express the neural marker B=tubulin, and occasionally contained pigmented cell nodules. Pax6 expression was significantly reduced in the retina of 3 mutant mice as compared to normal littermates.

Conclusions: Normal ocular development requires the function of the Ski proto-oncogene, and mice lacking Ski have many features associated with PHPV and Peter’s anomaly in humans. Defects in Ski-deficient mice closely resemble those described in animals lacking several of the retinoic acid receptor genes, or in animals exposed to excess retinoic acid during gestation. Ski has been shown to repress transcription induced by retinoic acid signaling, and may affect ocular development by regulating RA signaling. This may account for the reduced expression of Pax6 that we observed in Ski-deficient animals. Reduced Pax6 activity may also be responsible for the Peter’s phenotype as described in humans.
Extreme Hyperopia is the Result of Null Mutations in MFRP, which Encodes a Frizzled-Related Protein


Nanophthalmos is a rare disorder of eye development characterized by extreme hyperopia (farsightedness), with refractive error in the range of +8.00 to +25.00 diopters. Because the cornea and lens are normal in size and shape, hyperopia occurs because insufficient growth along the visual axis places these lensing components too close to the retina. Nanophthalmic eyes show considerable thickening of both the choroidal vascular bed and scleral coat, which provide nutritive and structural support for the retina. Thickening of these tissues is a general feature of axial hyperopia, whereas the opposite occurs in myopia. We have mapped recessive nanophthalmos to a unique locus at 11q23.3 and identified four independent mutations in MFRP, a gene that is selectively expressed in the eye and encodes a protein with homology to Tolloid proteases and the Wnt-binding domain of the Frizzled transmembrane receptors. This gene is not critical for retinal function, as patients entirely lacking MFRP can still have good refraction-corrected vision, produce clinically normal electro-retinograms, and show only modest anomalies in the dark adaptation of photoreceptors. MFRP appears primarily devoted to regulating axial length of the eye. It remains to be determined whether natural variation in its activity plays a role in common refractive errors.
Identifying Photoreceptors in Blind Eyes Caused by RPE65 Mutations: Prerequisite for Human Gene Therapy Success

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Mutations in RPE65, a gene essential to normal operation of the visual (retinoid) cycle, cause the childhood blindness known as Leber congenital amaurosis (LCA). Retinal gene therapy restores vision to blind canine and murine models of LCA. Gene therapy in blind humans with LCA from RPE65 mutations may also have potential for success but only if the retinal photoreceptor layer is intact, as in the early-disease stage-treated animals. Here, we use high-resolution in vivo microscopy to quantify photoreceptor layer thickness in the human disease to define the relationship of retinal structure to vision and determine the potential for gene therapy success. The normally cone photoreceptor-rich central retina and rod-rich regions were studied. Despite severely reduced cone vision, many RPE65-mutant retinas had near-normal central microstructure. Absent rod vision was associated with a detectable but thinned photoreceptor layer. We asked whether abnormally thinned RPE65-mutant retina with photoreceptor loss would respond to treatment. Gene therapy in RPE65(-/-) mice at advanced-disease stages, a more faithful mimic of the humans we studied, showed success but only in animals with better-preserved photoreceptor structure. The results indicate that identifying and then targeting retinal locations with retained photoreceptors will be a prerequisite for successful gene therapy in humans with RPE65 mutations and in other retinal degenerative disorders now moving from proof-of-concept studies toward clinical trials.
Mutation Screen of the Membrane-type Frizzled-Related Protein (MFRP) Gene in Patients with Inherited Retinal Degenerations

GJ Pauer, Q Xi, K Zhang, Elias I Traboulsi, Stephanie A Hagstrom

MFRP is a member of the frizzled-related protein family and contains a cysteine-rich domain essential for Wnt binding and signaling. MFRP is highly expressed in the retinal pigment epithelial cells of the eye. A splice donor mutation in the mouse ortholog of Mfrp is responsible for photoreceptor degeneration in the rd6 mouse. For these reasons, we investigated MFRP as a candidate gene for a phenotype associated with mutations. We screened 152 patients with inherited retinal degenerations including retinitis pigmentosa, Leber congenital amaurosis and Stargardt macular dystrophy. We identified five polymorphisms in the 5' untranslated region, four missense changes, six isocoding variants and four intronic changes. None of the sequence variants were interpreted as pathogenic.
Ocular Manifestations of Familial Adenomatous Polyposis (Gardner Syndrome)

Elias I Traboulsi

Familial adenomatous polyposis (FAP) is a colon cancer predisposition syndrome in which hundreds to thousands of precancerous colonic polyp become evident at a mean age of 16 years (range, 7-36 years). By age 35 years, 95% of patients have polyps. Gardner syndrome is the eponym given to a subgroup of FAP with extracolonic manifestations, such as pigmented ocular fundus lesions that resemble congenital hypertrophy of the retinal pigment epithelium, among others.
Optic Atrophy and Sensorineural Hearing Loss in a Family Caused by an R445H OPA1 Mutation

C Li, Gregory S Kosmorsky, K Zhang, BJ Katz, J Ge, Elias I Traboulsi

Autosomal dominant optic atrophy (ADOA) is the most common form of inherited optic atrophy. Four genetic loci have been associated with ADOA: OPA1, OPA2, OPA3, and OPA4. Out of these four loci, only one gene has been identified, OPA1. We previously described a unique syndrome of optic atrophy, sensorineural hearing loss, ptosis, and ophthalmoplegia in two unrelated families associated with an R445H mutation in OPA1. The R445H mutation is the only OPA1 mutation that has been associated with this syndrome. In this manuscript, we clinically characterize an unrelated family with four members affected by optic atrophy and hearing loss without extraocular motility abnormalities or ptosis. This family also harbors the R445H mutation. These cases help illustrate the intra- and inter-family variability in phenotype associated with this mutation. As we continue to learn more about OPA1 and the function of its protein product, we will begin to understand the pathophysiology of optic atrophy. This understanding will ultimately lead to novel treatments directed toward preventing the visual loss and disability associated with this inherited disease.
SOX2 Mutation Causes Anophthalmia, Hearing Loss, and Brain Anomalies

Stephanie A Hagstrom, GJ Pauer, J Reid, E Simpson, Susan Crowe, IH Maumenee, Elias I Traboulsi

The SOX2 transcription factor is expressed early in the embryonic stem cells of the blastocyst and later in the neural stem cells. It is a member of the SOX family of proteins that carry a DNA-binding high-mobility group domain and additional domains that regulate embryonic development and cell fate determinations. We surveyed 93 patients with severe eye malformations for mutations in SOX2. Here, we report a novel nonsense mutation in one female patient with bilateral clinical anophthalmia, absence of all optic pathways, and other neurological abnormalities. The mutation, Q155X, creates a premature termination codon early in the transcriptional activation domain and is likely to be a null allele. Our data show that mutations in SOX2 can cause not only anophthalmia, but also aplasia of the optic nerve, chiasm and optic tract, as well as modest bilateral sensorineural hearing loss, and global developmental delay, underscoring the importance of SOX2 in early human eye and brain development.
Study of the RAX Gene in Patients With Microphthalmia/Anophthalmia/Coloboma

N London, P Kessler, B Williams, GJ Pauer, Susan Crowe, Stephanie A Hagstrom, Elias I Traboulsi

Purpose: Microphthalmia, anophthalmia, and coloboma (MAC) are thought to have a significant genetic component. RAX is a homeobox gene that is expressed early in the developing retina and is important in retinal cell fate specification as well as in stem cell proliferation. Voronina et al. identified a single patient who was a compound heterozygote for autosomal recessive RAX mutations within a group of 75 anophthalmia and/or microphthalmia patients (Hum Mol Genet 2004; 13:315-322). These authors also reported two polymorphisms in the first and third exons. We screened a group of 25 MAC patients for RAX mutations.

Methods: We used standard PCR and automated sequencing techniques to amplify and sequence each of the three RAX exons. Patients’ charts were reviewed for clinical information.

Results: In addition to the polymorphisms described by Voronina et al., we identified a prevalent single nucleotide change (106 G>A) in the first exon that results in a glutamine to lysine alteration in the primary amino acid sequence. We further identified two other single nucleotide variations, also in the first exon, in two separate patients (153 A>C and 146 G>A), each resulting in an alteration in the primary sequence. We found no apparent correlation between the observed nucleotide variations and patient phenotype.

Conclusions: Sequence variations in RAX are common in our particular cohort of patients with major congenital ocular malformations and may play a role in the pathogenesis of these abnormalities.
Section 6

Ophthalmic Oncology
Choroidal Hemangioma

Arun D Singh, Peter K Kaiser, Jonathan E Sears

Choroidal hemangioma is an uncommon benign vascular tumor of the choroid that can be circumscribed or diffuse. Circumscribed choroidal hemangiomas are usually diagnosed between the second to fourth decade of life when they cause visual disturbances owing to the development of an exudative retinal detachment. Circumscribed tumors occur sporadically, without any associated local or systemic anomalies. Diffuse choroidal hemangiomas are usually evident at birth and generally occur as a part of neuro-oculo-cutaneous hemangiomatosis (Sturge-Weber syndrome).
Eccrine Hidrocystoma of the Eyelid

Arun D Singh, L McCloskey, MA Parsons, DN Slater

Aim: To report on the clinical features of eccrine hidrocystoma involving the eyelid.

Methods: Data on a series of consecutive patients with histopathologically confirmed diagnosis were reviewed.

Results: Among 34 patients, 69 tumours were identified. The mean age at diagnosis was 59 years (range 39-91 years). The majority (71%) of patients had only a single tumour. The tumours appeared as a small (median size=1 mm) clear cystic lesion with 87% located near the eyelid margin.

Conclusions: The eccrine hidrocystoma is a benign small cystic tumour that characteristically occurs close to but does not involve the eyelid margin.
Estimating The Risk of Malignant Transformation of a Choroidal Nevus

Arun D Singh, P Kalyani, A Topham

**Purpose:** To estimate the risk of malignant transformation of a choroidal nevus in the white population.

**Design:** Systematic literature review.

**Methods:** A literature review was performed to obtain data on the prevalence of choroidal nevi in the white population. Data from studies that used indirect ophthalmoscopy or otherwise corrected data to include the entire fundus were selected. Only studies reporting on the United States population were included. The number of affected individuals was estimated using 2000 U.S. census data. The estimate of annual incident choroidal melanoma cases in the corresponding age- and race-matched population was calculated using the Surveillance, Epidemiology, and End Result database (1973-2000). Average annual age-specific incidence rates for 1973 to 2000 for each of the 5-year age groups (adjusted for the U.S. 2000 population) were calculated and applied to the corresponding census data. The ratio of numbers of affected individuals with choroidal melanoma and choroidal nevi gave the annual rate of malignant transformation of a choroidal nevus.

**Main Outcomes Measures:** Annual rate of malignant transformation of a choroidal nevus in the white population of the U.S.

**Results:** The prevalence of choroidal nevus in the white U.S. population ranged from 4.6% to 7.9%. It was estimated that, on average, 8,864,625 individuals in the U.S. had a choroidal nevus. The number of individuals with choroidal melanoma in the corresponding age- and race-matched population ranged from 989 to 1,008 (mean, 1,002). The annual rate of malignant transformation of a choroidal nevus was estimated to be 1 in 8,845.

**Conclusions:** If it is assumed that all choroidal melanomas arise from preexisting nevi, then the published data suggest a low rate (1/8,845) of malignant transformation of a choroidal nevus in the U.S. white population.
Infrared Thermotherapy: From Laboratory to Clinic

HG Journee-de Korver, E Midena, Arun D Singh

Thermotherapy by the transpupillary route is an effective outpatient eye-salvaging therapy for intraocular tumors. It does not require surgery, it can be repeated, and it does not affect the healthy structures of the eye. Thermotherapy by the transscleral route is under investigation and may have potential in the treatment of choroidal melanomas. The indications for chemoreduction, thermochemotherapy, and thermotherapy for retinoblastoma remain under investigation.
Regression of Invasive Conjunctival Squamous Carcinoma in an HIV-positive Patient on Antiretroviral Therapy

S Holkar, HS Mudhar, A Jain, M Gupta, KE Rogstad, MA Parsons, Arun D Singh, IG Rennie

Case history of an African woman presenting with advanced HIV and a painful conjunctival lesion is presented. A conjunctival biopsy revealed invasive squamous cell carcinoma, with orbital invasion on computed tomography scan. She was commenced on antiretroviral therapy. She refused surgery to remove the eye and orbital contents (exenteration), and was referred to palliative care. Gradually, her immune status and ocular symptoms improved. At ophthalmic review, the tumour had apparently completely regressed. This unprecedented phenomenon may be due to antiretroviral therapy. Discussion covers conjunctival carcinoma and behaviour of HIV-related tumours with antiretroviral therapy. Antiretroviral drugs may offer a better alternative to disfiguring surgery in the future.
Uveal Melanoma: Epidemiologic Aspects

Arun D Singh, L Bergman, S Seregard

Melanomas of the ocular and adnexal structures comprise approximately 5% of all melanomas. The majority (85%) of ocular melanomas are uveal in origin; primary conjunctival and orbital melanomas are rare. The diagnosis of uveal melanoma is made by clinical examination including indirect ophthalmoscopy and by ancillary studies such as fluorescein angiography and ultrasonography.

Metastases to the liver develop within 15 years after the initial diagnosis and treatment in approximately 50% of patients with posterior uveal melanoma; however, clinically evident metastatic disease at the time of initial presentation is uncommon, indicating that there is early subclinical metastasis in most cases.
Section 7

Pediatrics and Strabismus
Adjustable Globe and Muscle Technique for Strabismus Repair in Thyroid Eye Disease

AJ Dal Canto, Susan Crowe, Julian D Perry, Elias I Traboulsi

**Purpose:** The purpose of this study is to review the outcomes of extraocular muscle (EOM) surgery in patients with thyroid eye disease (TED) using a technique in which the recessed muscle insertion is determined intraoperatively using adjustable globe and muscle positions.

**Methods:** 23 patients (5M:18F) aged 49.75 (20-71) yrs underwent strabismus surgery using an adjustable globe and muscle technique to treat diplopia. Diplopia followed orbital decompression in 7 patients, worsened after decompression in 3 patients, and occurred independently of decompression in 13. Six patients had ET (av. 27.3 pd); 7 had HT (av. 25.0 pd); 9 had a combination of ET and HT (av. 20.8 pd ET, 21.1 pd HT) and 1 had a combination of XT and HT (XT 10, HT 6). Ocular deviations were stable for an average of 6.5 months in 11 patients, and for more than 1 year in 12. Recession of BMR only was performed in 4 patients, IR only in 5 patients, BIR and BMR in 5 patients, combinations of uni- or bilateral IR and MR in 5 patients, RSR and LIR in 1 patient, RSR only in 1 patient, RIR, LSR, and RMR in 1 patient, and RLR and RIR in 1 patient. Results: Twenty of the 23 patients had excellent outcomes (no diplopia in primary and reading positions, without the use of prisms), and 3 had good outcomes (no diplopia in primary and reading positions with use of small prisms). Two patients underwent second surgeries to obtain their final outcomes (1 with excellent results and 1 with good results). Ten patients had less than 6 months of follow-up. Six patients kept their 6 month follow up visit, and 7 patients were seen 1 year or more after surgery. Linear regression did not show good correlation between the degree of strabismus and amount of recession required for eliminating diplopia (maximum R2 = 0.6697).

**Conclusions:** The adjustable globe and muscle technique provides reliable and consistent superior ocular alignment and relief from diplopia in the majority of patients with strabismus from TED. This technique may be preferable to table nomograms since poor correlation was obtained between the degree of strabismus and amount of recession. This is likely secondary to changes in the EOM (ie fibrosis) secondary to TED.
Complications of Cataract Extraction with or without Intraocular Lens Implantation in the First Two Years of Life

Susie Chang, David Huang, Susan Crowe, Elias I Traboulsi

Introduction: To report short-term complications of cataract extraction with or without intraocular lens (IOL) implantation in infants under two years of age.

Methods: Retrospective, case series. Thirty-eight eyes of 27 patients with unilateral (16 patients) or bilateral (11 patients) cataracts underwent cataract extraction before the age of 24 months. No specific criteria were used for inclusion in either the IOL group (22 eyes) or the non-implantation group (16 eyes). The decision to use an IOL depended on parental consent and on technical considerations at the time of cataract extraction. Surgery was performed through a limbal incision with primary posterior capsulotomy and anterior vitrectomy prior to capsular bag IOL implantation.

Results: No intraoperative complications occurred in any patient. Twenty-five of 38 eyes were operated before age 20 weeks. Follow-up ranged from 2 months to 6 years. Complications in the IOL group during this period include: 2 anterior chamber fibrin formation, 2 wound dehiscence (secondary to trauma), and the need for 6 additional surgeries predominantly to remove Elschnig pearls. In the aphakic group, complications include: 6 eyes (4 patients) with glaucoma, 5 additional surgeries, 1 endophthalmitis, and 1 retinal detachment.

Discussion/conclusions: IOL implantation in infants appears to be safe and may have a lower rate of glaucoma and other serious complications than cataract extraction without IOL implantation in the same age group. Additional studies are needed to confirm these observations.
Frequency of Intracranial Vascular Anomalies in Patients With Morning Glory Disc Anomaly

Phoebe D Lenhart, Amy K Hutchinson, Scott R Lambert, Elias I Traboulsi, Nancy J Newman, Valerie Biousse

Background or Purpose: Case reports have proposed an association between morning glory disc anomaly (MGDA) and intracranial vascular anomalies including moyamoya disease. The frequency of cerebrovascular abnormalities in children with MGDA is not known. We evaluated a series of patients with MGDA to ascertain the frequency of intracranial vascular anomalies.

Methods: Twenty patients followed for MGDA at two institutions were included in the study. We reviewed patients’ neuroimaging studies and neurological histories. We performed MRI/ MRA imaging of the brain on all patients who had not previously undergone neuroimaging.

Results: Six of 20 patients (30%) with MGDA had identifiable cerebrovascular anomalies. Three patients had abnormalities of the internal carotid artery and three had abnormalities of the anterior cerebral artery. These anomalies ranged from a small area of signal dropout within the proximal left A1 segment to bilateral stenosis of the internal carotid arteries. Three of the 20 patients had undergone revascularization procedures.

Discussion: We found intracranial vascular anomalies in 30% of patients with MGDA. It is uncertain whether these abnormalities represent benign isolated congenital anomalies or the earliest findings of progressive occlusive cerebrovascular disease.

Conclusions: We recommend that all patients with MGDA be imaged with MRI/ MRA. Computerized tomographic angiography (CTA) may be used to confirm and further delineate anomalies. Since the neurological consequences of moyamoya disease can be devastating, follow-up imaging may be indicated for patients with cerebrovascular anomalies, particularly if neurological signs or symptoms are present.
PHACE syndrome: Report of a Case with a Glioma of the Anterior Skull Base and Ocular Malformations

SB Cannady, TA Kahn, Elias I Traboulsi, PJ Koltai

PHACE syndrome consists of the constellation of manifestations including Posterior fossa anomalies of the brain (most commonly Dandy-Walker malformations), Hemangiomas of the face and scalp, Arterial abnormalities, Cardiac defects, and Eye anomalies. We present the case of a patient who presented with respiratory distress at birth secondary to a large nasal glioma. She was subsequently found to have a ventricular septal defect (VSD), a facial hemangioma, and a malformation of the eye and optic nerve head. The nasal glioma, which extended to the cribriform plate, has not been described in this syndrome. The tumor was resected through a coronal incision, midline nasal bone osteotomy, and a retrograde dissection from the nasal bones to the anterior skull base. Glioma of the skull base is a novel and serious manifestation of this uncommon condition.
Section 8

Refractive Surgery
Clinical Outcomes of the Investigational AcrySof® Angle-Supported Phakic Refractive IOL

Ronald R Krueger

**Purpose:** An evaluation of the safety and effectiveness of the AcrySof® Angle-Supported Phakic Refractive IOL when used for the correction of stable, high myopia.

**Methods:** Clinical trials were conducted in both the United States and Europe. In addition to presenting with stable high myopia, subjects were expected to meet minimum protocol criteria in endothelial cell density, anterior chamber depth, and preoperative astigmatism. The IOL was unilaterally implanted into the anterior chamber angle, with a follow-up period ranging from three to five years as specified in the protocol. Uncorrected Visual Acuity (UCVA), Predictability of Refraction, and Endothelial Cell Density Counts (ECC) are key endpoints that will be evaluated for the three study overview.

**Results:** US Phase 1, UCVA outcomes at 1 year postoperatively, demonstrated 100% (10/10) of subjects achieved visual acuity of 20/40 or better, while 70% (7/10) of subjects attained 20/20 or better. In the EU Phase 2 study at the 1 year mark, functional UCVA of 20/40 or better was demonstrated by 93.9% (92/98) of subjects, additionally, 46.9% (46/98) of subjects achieved 20/20 or better UCVA. High rates of predictability were evidenced by 100% (10/10) of US subjects, and 91% of EU subjects achieving MRSE within 1.0 D of their target refraction. At 1 year postoperatively, there were no incidences of endophthalmitis, pupil ovalization, persistently raised IOP, or ≥ 2 lines loss of BSCVA.

**Conclusions:** Clinical outcomes with the AcrySof® Phakic Refractive IOL indicate significant improvements in stable, high myopia, and high rates of functional uncorrected vision. The safety and effectiveness of this modality continue to be investigated in the U.S., Europe, and Canada.
Clinical Utility of Very High Frequency Ultrasound Imaging in Refractive Surgery

Ronald R Krueger, William J Dupps Jr

Purpose: To demonstrate 3 clinical examples in refractive surgery of essential diagnostic information available with very high frequency ultrasound.

Methods: Very high frequency ultrasound imaging (Artemis, UltraLink LLC, St. Petersburg, FL) was used to analyze corneal or anterior chamber (AC) dimensions in patients being evaluated before and/or after refractive surgery. The ultrasonic frequency of analysis was 50 MHz (interface resolution of 35 µm, precision of 1-5 µm) and the 3D scanning was performed with water immersion. Corneal scans with 3D mapping (ArtPro software) of corneal, flap and epithelial thickness profiles was performed following LASIK flap creation with the IntraLase laser (Irvine, CA) vs Moria M2 microkeratome (Antony, France). The same corneal analysis was performed in the consult evaluation of an optically aberrated eye following LASIK with presumed decentration. Finally, meridional sections of the AC were viewed to determine angle to angle AC diameter in comparison to the white to white (W2W) diameter of the horizontal cornea as a sizing metric for AC phakic IOL implantation.

Results: The IntraLase flap thickness profile (90 µm depth) showed a uniform thickness of 101 µm centrally, 103 µm superiorly, 113 µm nasally, 103 µm inferiorly and 98 µm temporally at distances 3mm from center, while the Moria M2 (90 head) showed a variable thickness profile of 75 µm centrally, 165 µm superiorly, 167 µm nasally, 116 µm inferiorly and 147 µm temporally. The eye with a presumed decentration after LASIK with inferotemporal (IT) topographic steepening vs superonasal (SN) flattening and inferotemporal myopic coma by wavefront of 1.82 µm (6.5 µm pupil), revealed a corneal thickness of 481 µm at 3mm IT and 563 µm at 3 mm SN, suggestive of ectasia rather than decentration. Finally, 3 eyes with a W2W of 11.7 mm, 12.5 mm and 11.8 mm had AC diameters of 12.0 mm, 12.5 mm and 12.2 mm, which aided in the sizing of 3 AC angle supported phakic IOLs with good lens vaulting in each case.

Conclusion: Very high frequency ultrasound is a useful diagnostic tool for studying the microanatomy of the eye and determining critical measurements used in refractive surgery.
Indications for Surface Ablation Vision Correction

Marcelo V Netto, Steven E Wilson

Laser in situ keratomileusis (LASIK) remains the dominant procedure in refractive surgery. Its popularity is primarily due to the maintenance of an intact epithelium over the central cornea, resulting in reduced postoperative discomfort, faster visual rehabilitation, and a reduced healing response resulting in less regression for higher corrections compared to surface ablation procedures such as photorefractive keratectomy (PRK) and laser subepithelial keratomileusis with a manual (LASEK) or mechanical epithelial lift (Epi-LASIK). However, there are situations where surface ablation procedures continue to be an excellent choice and, in some cases, the procedure of choice. These indications are reviewed in this article.
U.S. Clinical Outcomes of the Investigational AcrySof® Angle-Supported Phakic Refractive IOL

Ronald R Krueger

**Purpose:** An evaluation of the safety and effectiveness of the AcrySof® Angle-Supported Phakic Refractive IOL when used for the correction of stable, high myopia.

**Setting:** U.S. Clinical Trial

**Methods:** This U.S. clinical investigation of the AcrySof® Phakic IOL incorporated an open label, single arm, non-randomized study design. Adult subjects between 18 and 49 years of age with stable, high myopia, and otherwise healthy eyes were eligible for inclusion. Subjects were excluded from participation for anterior chamber depth less than 3.2 mm, astigmatism greater than 2.0 D, mesopic pupil diameter greater than 7.0 mm, or endothelial cell density not meeting protocol criteria per age. The foldable, single-piece AcrySof® Phakic IOL was unilaterally implanted into the anterior chamber via the Monarch® II Injector. Uncorrected and Best Spectacle-Corrected Visual Acuity (UCVA & BSCVA), Predictability of Refraction, and Maintenance of Best Spectacle-Corrected VA (BSCVA) were some of the primary endpoints evaluated for safety and effectiveness.

**Results:** UCVA outcomes at 1 year postoperatively demonstrated 100% (10/10) of subjects achieved visual acuity of 20/40 or better, while 70% (7/10) of subjects attained 20/20 or better. BSCVA results captured at 1 year postoperatively indicated 100% of subjects achieved BSCVA of 20/40 or better, and 100% reached 20/20 or better. High rates of predictability were evidenced by 90% (9/10) of subjects achieving MRSE within 0.5 D of their target refraction, and 100% (10/10) of subjects being within 1.0 D of target refraction. Sixty percent (6/10) of subjects gained 1 line of BSCVA, 30% (3/10) demonstrated no change, and no subjects presented with a decrease of 2 or more lines.

**Conclusions:** Preliminary clinical outcomes with the AcrySof® Phakic Refractive IOL indicate significant improvements in stable, high myopia. Investigations continue for examination of safety and effectiveness.
Wavefront Analysis in Normal Refractive Surgery Candidates

Marcelo V Netto, R Ambrósio, TT Shen, Steven E Wilson

**Purpose:** To quantify the higher order aberrations of refractive surgery candidates and compare the wavefront-determined refractions with manifest refractions refined with a ± 0.25 Jackson cross cylinder.

**Methods:** Results of 226 consecutive patients (418 eyes) were analyzed with the WaveScan WavePrint system (VISX, Santa Clara, Calif). Only patients with normal eyes without previous surgery were included.

**Results:** The mean spherical equivalent refraction determined with wavefront analysis was -3.40 ± 3.14 diopters (D) (range: -10.72 to +5.41 D). The largest amount of higher order aberrations was detected with a 6-mm pupil diameter (coma 0.14 ± 0.08 μm; trefoil 0.10 ± 0.07 μm; spherical aberrations 0.09 ± 0.07 μm). The mean root-mean-square of higher order aberrations and total aberrations were 0.23 ± 0.11 μm and 4.00 ± 2.45 μm, respectively. No statistically significant correlation was noted between higher order aberrations and gender (P = 0.7) or between higher order aberration and refractive level (P > .59). The mean differences in spherical equivalent refraction, sphere, and cylinder between WaveScan measurements and manifest refraction were 0.36 ± 0.41 D, 0.40 ± 0.44 D, and 0.28 ± 0.32 D, respectively.

**Conclusions:** This study provides reference values for higher order aberrations in normal refractive surgery candidates. Wavefront analysis also proved to be a valuable tool for objectively measuring preoperative refractive error.
Wavefront-guided Surface Ablation with Prophylactic Use of Mitomycin C after a Buttonhole Laser in situ Keratomileusis Flap

Maria R Chalita, Alan S Roth, Ronald R Krueger

**Purpose:** To describe the surgical outcome of a patient who had a previous buttonhole after laser in situ keratomileusis (LASIK) and 3 months later, had wavefront-guided photorefractive keratectomy (PRK) with topical mitomycin C 0.02%.

**Methods:** A 38-year-old man underwent bilateral LASIK for correction of myopic astigmatism. A buttonhole in his right eye LASIK flap occurred, but the surgeon decided to proceed with ablation due to the small size of the buttonhole. After LASIK, the patient complained of monocular diplopia in his right eye with 20/30 best spectacle-corrected visual acuity. Wavefront analysis showed a large amount of higher order aberrations, especially coma. Slit-lamp examination revealed a moderate buttonhole scar. Three months after LASIK, the patient underwent wavefront-guided PRK with application of topical mitomycin C 0.02% on the stromal bed, for a duration of 2 minutes.

**Results:** One month after wavefront-guided PRK, his uncorrected visual acuity was 20/25 in the right eye, with no symptoms. Best spectacle-corrected visual acuity in the right eye was 20/15 with +0.25 -0.50 x 110 degrees. No haze or scar was seen on slit-lamp examination. Wavefront analysis showed a decrease in higher order aberrations, especially coma and spherical aberration.

**Conclusions:** Wavefront-guided PRK with prophylactic topical mitomycin C was effective in treating a patient with visual symptoms and loss of BSCVA after a LASIK flap buttonhole. No delayed epithelial healing, side effects or complications were noted due to mitomycin C.
Wavefront-Guided LASIK Retreatment for Residual Myopia and Aberrations in Symptomatic Post-LASIK Eyes

Ronald R Krueger, Maria R Chalita, Marcelo V Netto, Meng Xu

Precis: Aberrations and diminished visual quality after LASIK can be improved with wavefront-guided LASIK.

Purpose: Evaluate the outcome of wavefront-guided LASIK after symptomatic standard LASIK.

Methods: Thirty eyes underwent wavefront-guided reablation with Alcon CustomCornea. Complete exam including LADARWave mapping was done preop, 1 week and 3 months postop. Statistical analysis was performed using paired T-test.

Results: All eyes had an improvement in symptoms. One showed a decrease in total aberrations (4.28 µm to 1.55 µm), and high order aberrations (1.09 µm to 0.96 µm). Three months postop UCVA was >20/20 in 33.33% and BCVA was >20/20 in 77.77%.

Conclusion: Wavefront-guided LASIK retreatment represents a viable option for symptomatic post LASIK eyes.
Wound Healing in the Cornea: A Review of Refractive Surgery Complications and New Prospects for Therapy

*Marcelo V Netto, RR Mohan, Steven E Wilson*

**Purpose:** The corneal wound healing response is of particular relevance for refractive surgical procedures since it is a major determinant of efficacy and safety. The purpose of this review is to provide an overview of the healing response in refractive surgery procedures.

**Methods:** Literature review.

**Results:** LASIK and PRK are the most common refractive procedures; however, alternative techniques, including LASEK, PRK with mitomycin C, and Epi-LASIK, have been developed in an attempt to overcome common complications. Clinical outcomes and a number of common complications are directly related to the healing process and the unpredictable nature of the associated corneal cellular response. These complications include overcorrection, undercorrection, regression, corneal stroma opacification, and many other side effects that have their roots in the biologic response to surgery. The corneal epithelium, stroma, nerves, inflammatory cells, and lacrimal glands are the main tissues and organs involved in the wound healing response to corneal surgical procedures. Complex cellular interactions mediated by cytokines and growth factors occur among the cells of the cornea, resulting in a highly variable biologic response. Among the best characterized processes are keratocyte apoptosis, keratocyte necrosis, keratocyte proliferation, migration of inflammatory cells, and myofibroblast generation. These cellular interactions are involved in extracellular matrix reorganization, stromal remodeling, wound contraction, and several other responses to surgical injury.

**Conclusions:** A better understanding of the complete cascade of events involved in the corneal wound healing process and anomalies that lead to complications is critical to improve the efficacy and safety of refractive surgical procedures. Recent advances in understanding the biologic and molecular processes that contribute to the healing response bring hope that safe and effective pharmacologic modulators of the corneal wound healing response may soon be developed.
Section 9

Retina
A Phase I, Open-Label, Dose Escalation Trial of Intravitreal Injection of Small Interfering RNA Molecule in Subjects with Neovascular AMD

E Quinlan, Q Nguyen, Peter K Kaiser, et al

**Purpose:** RNA interference (RNAi)-based therapies silence genes via degradation of the desired mRNA. RNAi is a natural process where double-stranded RNA (dsRNA) destroys mRNAs whose sequences are homologous to the dsRNA resulting in specific gene silencing. This approach can block production of the VEGF receptor 1 by blocking VEGFR1 mRNA production (Sirna-027, Sirna Therapeutics/Allergan).

**Methods:** Preclinical studies Sirna-027 reduced the levels of VEGFR1 in vitro and in vivo in a murine model of CNV by 40% to 57% and decreased the extent of CNV by 45% to 66%, depending on the route of administration (Shen et al., 2005). A Phase I dose-escalating clinical trial of Sirna-027 (100 μg – 1600 μg) in patients with AMD was performed.

**Results:** Single doses of Sirna-027 were safe and well tolerated. Of the 23 patients with AMD enrolled so far, all experienced stabilization of VA, and 23% experienced visual improvement of 3 or more lines of VA within 8 weeks of Sirna-027 injection. No serious adverse events or dose-limiting toxicities were observed. A phase II, randomized, multi-dose clinical trial is expected to begin in 1Q 2006.

**Conclusions:** Sirna-027 reduces VEGFR1 mRNA and the receptor itself in preclinical studies. Early phase I studies show the drug is safe on intravitreal injection with efficacy in subfoveal CNV due to age-related macular degeneration. Further studies are ongoing to explore the magnitude of this effect.
Abnormal Distribution of Red/Green Cone Opsins in a Patient with an Autosomal Dominant Cone Dystrophy

Vera L Bonilha, Joe G Hollyfield, S Grover, GA Fishman

The purpose was to define the distribution of the red/green and blue opsins in cones from donor eyes from an affected member of a clinically well-characterized family with an autosomal dominant form of cone dystrophy. Tissue was fixed and processed for immunohistochemistry. Cryosections were studied by indirect immunofluorescence, using well-characterized antibodies to cone cytoplasm, rhodopsin, and cone opsins. The cone-associated matrix was also labeled with the lectin PNA. The affected donor eyes were compared to a postmortem matched normal eye. The results showed that electroretinogram (ERG) testing three years prior to the affected member’s death showed normal rod function, while the cone b-wave amplitude was reduced 40% below the lower limit of normal. Fundus exam showed only isolated drusen within the macula. Either a normal-appearing or only nonspecific macular findings were noted in the other affected family members who were examined. Immunofluorescence studies showed that blue cone opsin was restricted to the outer segments of blue cones in the affected retina. Red/green opsins were distributed along the entire plasma membrane of these cone types, from the tip of the outer segment to the synaptic base. Cone-associated matrix displayed a heterogeneous distribution. These patterns were observed both in the macula and in the periphery of the affected retina. Cone pedicles appeared larger than normal. In contrast, rhodopsin staining appeared normal. The immunocytochemical data obtained here suggest that the clinical manifestation of this dystrophy is associated with an abnormal distribution of cone red/green opsins. Additionally, changes in the cone pedicles could have contributed to the abnormal cone ERG in this patient.
Anecortave Acetate 15 mg Suspension for the Treatment of Exudative Age-Related Macular Degeneration

Peter K Kaiser, J Slakter

**Purpose:** Anecortave acetate is a cortisene that inhibits angiogenesis, but has minimal glucocorticoid (anti-inflammatory) or mineralocorticoid (salt-retaining) activity. Anecortave inhibits angiogenesis downstream from VEGF, and thus can inhibit angiogenesis driven by multiple stimuli.

**Methods:** Anecortave is administered by posterior juxtascleral depot administration using a specially designed blunt cannula.

**Results:** The C-98-03 Study was a monotherapy study of anecortave versus placebo. Of the 128 patients included, anecortave was statistically superior to placebo for < 3 line change, prevention of vision loss, and suppression of CNV growth. The C-00-07 Study evaluated anecortave versus placebo following verteporfin PDT. In the 136 patients included, patients who received anecortave and PDT maintained better vision than patients who received PDT alone. The C-01-99 study evaluated anecortave versus verteporfin PDT for predominantly classic CNV. In the 511 patients enrolled, using the FDA pre-defined 7% 95% CI, statistical non-inferiority was not met. However, using the more clinically relevant CI for PC CNV (14%), the primary outcome was met. The C-02-60 is a 48 month study of anecortave versus placebo to determine whether anecortave reduces the risk of CNV developing in eyes with dry AMD. Anecortave and the administration procedure have been shown to have an excellent safety profile. Most adverse effects observed in clinical studies have been mild and transient, and occurred at a similar rate as placebo.

**Conclusions:** Anecortave is safe and effective in the management of subfoveal CNV due to AMD. Further studies are ongoing.
Annexins (also known as lipocortins) are a family of calcium and phospholipid-binding proteins. At least 20 members of this family are known, and they have a wide range of potential functions, such as vesicular transport and trafficking, endocytosis, exocytosis and cell-cell adhesion. Annexins have molecular weights ranging between 30 and 40 kDa (the exception is annexin VI which is 66 kDa) and possess striking structural features. To qualify as an annexin, a protein must have 1) the presence of a conserved 70 amino acid domain repeated either 4 or 8 times in the overall structure (annexin VI has an 8 repeating amino acid domain; whereas the rest have 4), 2) the ability to bind phospholipids in the presence of calcium. Annexins are exported from the cytosol to the exterior of cells across the plasma membrane by an unknown mechanism. When located extracellular, some annexins have been shown to function as receptors for other extracellular proteins: annexin II binds to tenascin and tissue plasminogen activator, while annexin V binds to collagen. Several annexins were identified in a recent proteomic study of drusen. Specifically, peptides from annexins I, II, IV, and VI were found by LC MS/MS Q-Tof analysis of trypsin digested drusen proteins. To define the precise the distribution of these annexins in drusen and Bruch’s membrane/choroid interface, we conducted immunocytochemical studies using a series of commercially available annexin antibodies.
Biomarker Discovery for Age-Related Macular Degeneration

John W Crabb, J Gu, Xiarong Gu, John S Crabb, E Bala, G Sturgill, E Simpson, Neal S Peachey, Stephanie A Hagstrom, Jonathan E Sears, Peter K Kaiser, Hilel Lewis, S Yaniglos, RG Salomon

Purpose: To develop a blood test for age-related macular degeneration (AMD) that will allow identification of those at risk prior to clinical evidence of the disease.

Methods: Blood was collected from clinically documented AMD and age-matched normal, healthy donors at the Cole Eye Institute, Cleveland Clinic Foundation and Louis Stokes Cleveland VA Medical Center. Plasma carboxyethylpyrrole (CEP) immunoreactivity and CEP autoantibody titer were determined by ELISA. Plasma was prefractionated on reversed phase or anti-CEP antibody coated magnetic beads then analyzed by high resolution MALDI TOF mass spectrometry to detect peptides. Logistic regression modeling of ELISA data for the c-statistic and odds ratio was performed with SAS/STAT software. Cluster analysis and cross validation of peptidomic patterns was performed with Gene-Spring software.

Results: ELISA analyses of plasma from AMD and normal control donors extended our preliminary report (2003 J Biol Chem 278, 42027) and confirmed that AMD donors (n = 275) exhibit overall higher mean levels of CEP immunoreactivity (1.6x) and autoantibody titer (1.4x) relative to age-matched normal donors (n = 105), including early stage AREDS AMD category 2 donors. MALDI TOF mass spectrometric analyses of CEP immunoaffinity fractionated plasma allowed correct prediction of 57 of 60 plasma (95%) as either AMD or normal based on peptidomic profiles.

Conclusions: Plasma CEP immunoreactivity and autoantibody titer are typically elevated in AMD patients. Plasma peptidomic patterns may provide a method for early identification of individuals susceptible to developing AMD, before retinal degeneration.
Bipolar Specific Expression of Nyctalopin Fusion Gene Rescues no-b wave Phenotype in nob Mice

RG Gregg, MA McCall, Neal S Peachey

Purpose: The nob mouse lacks the b-wave component of the ERG. This phenotype results from a molecular defect, a deletion in the nyx gene that encodes a protein called nyctalopin. The expression pattern of the nyctalopin protein in the retina is not unequivocally established, although the ERG phenotype suggests an ON bipolar localization. To test this hypothesis, we constructed transgenic mice and crossed them to nob mice and examined the ERGs in nob/Y transgenic male offspring.

Methods: We expressed an enhanced yellow fluorescent protein (EYFP): nyctalopin fusion cDNA under the control of the GABAC rho1 promoter, which directs expression specifically to bipolar cells. Transgenic mice were generated by conventional methods. Twelve founders were identified and, to date, we have identified 2 lines that transmit and express the transgene. These mice then were crossed to nob mice. Genotypes were determined by PCR and mice that carried both nob and the transgene identified. ERGs were recorded from transgenic/nob and nob mice under ketamine and xylazine anesthesia. Retinas were immersion fixed and prepared for sectioning and immunohistochemistry using standard techniques. Cryostat sections were cut and sections reacted with a variety of markers to localize expression of EYFP:nyctalopin positive cells.

Results: The expression of the EYFP:nyctalopin fusion protein in bipolar cells restores the ERG b-wave. Immunohistochemistry shows that the fusion protein is located in the OPL at the tips of the ON bipolar cells. A full light- and dark-adapted ERG series will be completed once additional mice have been generated by breeding.

Conclusions: Expression of nyctalopin in bipolar cells can rescue the ERG in the nob mice. Thus, the absence of nyctalopin expression on bipolar cell dendrites in the OPL is the primary defect in nob retinal processing.
Characterization of Semenogelin Proteins in the Human Retina

Vera L Bonilha, Mary E Rayborn, Karen G Shadrach, Åke Lundwall, Johan Malm, Sanjoy K Bhattacharya, John W Crabb, Joe G Hollyfield

Semenogelin I and II are the major proteins present in semen coagulum. In the present study, semenogelin I and II were detected in human RPE lysates by proteomic analysis. We further analyzed the expression of these proteins in the retinal cells in vivo and in vitro. Western blots detected semenogelin I and II in both RPE and neural retina while the vitreous contained only semenogelin II. Cryo and paraffin sections of human retina were processed for both immunoﬂuorescence and DAB reaction with an antibody that recognizes both forms of semenogelin proteins. Retina and RPE total lysates were evaluated for the presence of these proteins and in a human RPE cell line (D407). Both proteins were detected by western blot in human RPE and in D407 cell lysates. Immunoreactivity was detected in the ganglion cell and photoreceptor layer of the retina. Our data support the expression of semenogelin I and II in the human retina in several different compartments. Further studies toward addressing the function of these proteins in the retina are in progress.
Contribution of Calcium Channel β Subunits to the Mouse dc-Electroretinogram

J Wu, AD Marmorstein, H Shin, RG Gregg, Neal S Peachey

Purpose: The electroretinogram (ERG) represents the combination of several distinct cellular processes and conductances generated by the neural retina or the retinal pigment epithelium (RPE). Of the latter, the light peak (LP) is known to reflect a Cl conductance across the basal membrane of the RPE. This conductance is thought to be Ca\(^{2+}\) sensitive. Since nimodipine, which blocks L-type Ca\(^{2+}\) channels, attenuates the LP in rats, we recorded ERGs in mice lacking each of the 4 β subunits that are critical for correct localization and function of Ca\(^{2+}\) channels.

Methods: Mice lacking one of the four known Ca\(^{2+}\) channel β subunits have been generated by gene targeting and transgenic rescue (CNS-β\(_1\), CNS-β\(_2\)), by gene targeting alone (β\(_3\)), or arose spontaneously (lethargic, β\(_4\) mutant). After overnight dark adaptation, mice were anesthetized with ketamine and xylazine, the pupils were dilated, and an ERG was recorded to a 7-min stimulus. Intensity-response functions were developed from recordings made on separate days.

Results: There were no significant differences in any ERG component generated by the RPE in CNS-β\(_1\), CNS-β\(_2\) or β\(_3\) KO mice. In comparison to control littermates, the LP was significantly decreased in lethargic mice.

Conclusions: These results are consistent with a model for LP generation that includes a modulatory role of L-type Ca\(^{2+}\) channels on a Ca\(^{2+}\)-sensitive Cl channel that underlies the LP, and implicate the β\(_4\) subunit as being specifically involved. The unique role of β\(_4\) in the RPE resembles the role of β\(_2\) in VDCC-controlled release of glutamate at the photoreceptor-to-bipolar cell synapse (Ball et al., IOVS, 2002;43:1595).
Crystallin Distribution in Bruch’s Membrane-Choroid Complex from AMD and Age-Matched Donor Eyes

Ko Nakata, John W Crabb, Joe G Hollyfield

Crystallins were consistently found in a recent proteomic analysis of drusen from age-related macular degeneration (AMD) donor eyes. Here we compare the distribution of several crystallins in drusen, Bruch’s membrane and choroid from AMD and non-AMD age-matched control eyes. Immunohistochemistry and Western blots of tissue samples were performed using antibodies to aA-, aB- and bB1-crystallins. Bruch’s membrane, drusen and the subjacent choroidal connective tissue from AMD tissues showed greater immunoreactivity for aA- and aB-crystallins than were observed in normal age matched control tissues. Western blots also demonstrated more intense aA- and aB-crystallin signals from AMD tissues than were present in age-matched controls. In contrast, bB1-crystallin was not observed in Western blots and was evident only at background levels in both AMD and age-matched control tissues. These data indicate that aA- and aB-crystallins accumulate in Bruch’s membrane and choroidal connective tissues to a greater degree in AMD than in normal aging.

These findings suggest that the accumulation of these small heat shock proteins at this critical interface below the RPE reflects a disease-related stress response manifested during the progression of AMD.
Effects of Large Gene Mutation in the Veils Mouse Retina

BS Lee, S Kameya, RS Smith, W Hicks, J Hsu, Neal S Peachey, JK Naggert, PM Nishina

**Purpose:** *Large* encodes for a putative glycosyltransferase whose only known substrate is alpha-dystroglycan, a component of the dystrophin glycoprotein complex (DGC). Mutations in DGC components are known to cause human disorders including muscular dystrophies, and ocular and other brain related abnormalities. The veils mutant (*Large*$_{vls}$) is a remutation of *Large*$_{myd}$. Both mutations show very similar phenotypes. Effects of the *Large*$_{myd}$ mutation in mouse muscle and brain have been well documented. There is, however, little information on the effects of either *Large* gene mutations on the retina. Here we report a comprehensive characterization of retinal abnormalities in both *Large*$_{vls}$ and *Large*$_{myd}$ mutant mice.

**Methods:** Retinal structure was studied using light and electron microscopy (EM) and immunohistochemistry. Retinal function was assessed using electroretinograms (ERGs) recorded under dark- and light-adapted conditions. Results obtained from *Large* mutants were compared to unaffected control littermates.

**Results:** In both *Large*$_{vls}$ and *Large*$_{myd}$ mice, the inner limiting membrane (ILM) is disrupted and ganglion cells are displaced ectopically into the vitreous. In both mutants, there is an age-related loss of cells in the peripheral retina. Unlike muscle, most components of DGC were significantly reduced in the ILM. In the outer plexiform layer (OPL), however, the DGC is intact although beta-dystroglycan expression is reduced. At the EM level, the integrity of the OPL in *Large* mutant mice was compromised and synaptic complexes were missing or disrupted. Under all stimulus conditions, the ERG b-wave was dramatically slowed and reduced in amplitude in both *Large*$_{vls}$ and *Large*$_{myd}$ mice.

**Conclusions:** The results indicate that mutations in *Large* cause a number of retinal abnormalities and indicate that proper glycosylation of alpha-dystroglycan is important for maintaining the structural and functional integrity of the mouse retina. It remains to be determined whether normal DGCs are never formed or if they are formed and then rapidly degraded. In either case, EM and ERG data indicate that alpha-dystroglycan and other DGC components are required to form and maintain ribbon synapses in the OPL. Differences between muscle and retina indicate that components of DGC may be regulated differently in the retina.
Expression of Tulp1 During Normal Mouse Development

GJ Pauer, Q Xi, Mary E Rayborn, Joe G Hollyfield, J Wu, Neal S Peachey, Stephanie A Hagstrom

**Purpose:** TULP1, a member of a family of four proteins with unknown function designated tubby-like proteins or TULPs, is expressed specifically in photoreceptor cells. Mutations in *TULP1* are associated with autosomal recessive retinitis pigmentosa and Tulp1 knockout mice develop an early-onset, progressive photoreceptor degeneration. To develop a body of information regarding mouse Tulp1, we analyzed the expression of mouse Tulp1 mRNA and protein during development and examined the retinal anatomy and function in young *tulp1*-/− mice.

**Methods:** Tulp1 mRNA expression in wild-type (WT) mice was studied with quantitative RT-PCR while protein expression was observed using Western blot analysis and immunohistochemistry. Retinal function and anatomy in *tulp1*-/− mice was studied using electroretinography (ERG) and histology.

**Results:** In WT retinas, Tulp1 mRNA was first detected at postnatal day (P) 1 and then increased incrementally to a stable peak at P11. Tulp1 protein expression paralleled the mRNA expression. At P15, the rod-mediated ERGs of *tulp1*-/− mice were reduced in amplitude, while cone-mediated ERGs were comparable to those recorded from WT littermates. Histologically at P15, the outer nuclear layer (ONL) of *tulp1*-/− retinas was normal in thickness, but the outer segments appeared slightly shorter than WT littermate controls. Small gaps near the outer limiting membrane were also seen in the ONL of *tulp1*-/− retinas that were not detected in the WT retinas.

**Conclusions:** Tulp1 mRNA and protein are both detected in the developing mouse eye immediately following birth. In *tulp1*-/− mice, photoreceptor degeneration is apparent at an earlier age (P15) than previously described. At this early stage of photoreceptor degeneration, rod function is reduced to a greater extent than predicted from the anatomical presentation. In addition, cone function appears to be spared indicating that cones may be more resilient to the lack of Tulp1 and/or they degenerate at a later age due to an undefined secondary effect. These results indicate a fundamental role for TULP1 in maintaining the viability of rod photoreceptor cells.
Fractionation of Retina for Proteomic Analysis

John S Crabb, K Renganathan, Xiaorong Gu, K West, Sanjoy K Bhattacharya, Z Wu, John W Crabb

Purpose: Detection of integral membrane proteins by 2D-PAGE can be problematic because proteins like rhodopsin usually remain embedded in the first dimension immobilized pH gradient. Here we explore the efficacy of solution state isoelectricfocusing (IEF) and one-dimensional SDS polyacrylamide gel electrophoresis (1D PAGE) for fractionation of bovine retina for proteomic analysis.

Methods: Bovine retinal protein extraction efficiency was evaluated in 7M urea and 2M thiourea containing one of eight different detergent solutions. Rhodopsin extraction efficiency was determined by Western analysis. Retinal protein recovery following solution state IEF in 3% ASB-14, 4% CHAPS, or 3% dodecyl maltoside was quantified by the Bradford and bicinchoninic assays. Following solution state IEF using the Multicompartment Electrolyzer (MCE, Proteome Systems) and 1D PAGE, bands were excised, digested in situ with trypsin and proteins identified by capillary LC MS/MS.

Results: Retinal extracts in 3% ASB-14 yielded less high mass aggregates of rhodopsin and greater protein recovery in IEF. Solution state IEF in 3% ASB-14 separated retinal proteins into pI fractions 3-5, 5-6.5, 6.5-8, and 8-11. After IEF, 1D-PAGE revealed different electrophoretic profiles for each trapping chamber fraction. A total of 352 proteins were identified from mass spectrometric analyses of select 1D gel bands, including rhodopsin, 31 other integral membrane proteins and 33 membrane-associated proteins.

Conclusions: For proteomic studies of retina, solution state IEF followed by 1D-PAGE provides an attractive alternative to 2D gel analyses with immobilized pH gradients. In this study, ASB-14 was the most effective detergent for the solubilization and extraction of retina and identification of rhodopsin.
Gene Expression Profiling in *Tulp1*-/− Mice

K Bollinger, GJ Pauer, Stephanie A Hagstrom

**Purpose:** TULP1 is a member of the TULP family of proteins with unknown function and is expressed specifically in photoreceptor cells. Mutations in *TULP1* cause autosomal recessive retinitis pigmentosa and Tulp1 knockout mice develop an early-onset, progressive photoreceptor degeneration. Our previous data indicate that TULP1 may be involved in the movement of proteins from the inner segment to the outer segment of photoreceptors. To better understand the pathogenic mechanism and potential pathways involved in photoreceptor degeneration in *tulp1*-/− mice, we are comparing the gene expression profile in the *tulp1*-/− retinas to wild-type (WT) retinas.

**Methods:** RNA was isolated from the retinas of *tulp1*-/− and WT littermate mice at P15. The RNA was used to make biotinylated cRNA for gene chip hybridization. cRNA was hybridized to Mouse Genome 430 2.0 arrays (Affymetrix) containing ~45,000 probe sets representing ~39,000 transcripts. Scanning of probe arrays and data analysis were performed according to Affymetrix protocols. Only the transcripts between experimental groups that have more than a 2-fold change and a p-value less than 0.005 are reported.

**Results:** We identified 97 transcripts that were significantly altered in the *tulp1*-/− retinas as compared to WT retinas. For transcripts that were decreased in *tulp1*-/− retinas, the biological processes they are associated with include membrane trafficking, cargo binding and dynein-based movement. The majority of transcripts that were increased in *tulp1*-/− retinas were unknown, novel cDNAs. We are currently using real-time RT-PCR to verify and quantify our array data and immunohistochemistry to examine spatiotemporal changes in expression patterns.

**Conclusions:** Lack of Tulp1 changes the gene expression profile of the retina. Our results indicate that transcripts involved in vesicular movement are down-regulated in *tulp1*-/− retinas and suggest that photoreceptor degeneration in the *tulp1*-/− mouse may be due to a disruption of the intracellular protein transport pathway.
Glucose Utilization by the Retinal Pigment Epithelium: Evidence for Rapid Uptake and Storage in Glycogen, Followed by Glycogen Utilization

Preenie deS Senanayake, Anthony Calabro, Jane G Hu, Vera L Bonilha, Aniq Darr, Dean Bok, Joe G Hollyfield

Glucose utilization and glycogen metabolism by human retinal pigment epithelium (RPE) cultures with high transepithelial resistance maintained on porous Millicell polycarbonate filters were quantified by fluorophore-assisted carbohydrate electrophoresis (FACE). Glucose uptake was more efficient at the apical surface of the RPE. The utilization of glucose when restricted to either the apical or basal medium was also evaluated. Under both conditions, glucose was quickly transported to the opposite compartment and rapidly utilized. However, glucose from the apical compartment was depleted to a greater extent than from the basal compartment. The de novo synthesis and accumulation of glycogen accompanied glucose utilization. This was paralleled by a concomitant increase in lysosomal glycogen degradation measured as an increase in cell-associated maltodextrins. The highest levels of glucose in glycogen and maltodextrins occurred at 24 hours, declining to basal levels at 72 hours. Glucose transporter expression in the RPE cultures was evaluated with the reverse transcriptase-polymerase chain reaction. Glucose transporter-1 (GLUT 1) was the isoform expressed in these cells. GLUT 1 localization was determined by immunocytochemistry. GLUT 1 localizes to the apical and basolateral border of the RPE. The intensity of fluorescence was higher on the apical border. The rapid depletion of medium glucose suggests that RPE culture studies should replenish medium glucose more frequently than every 72 hours to maintain physiologically relevant glucose concentrations. These studies are the first to demonstrate glucose, glycogen and maltodextrin metabolism by RPE cells, and their detection and quantitation by FACE.
Identification of High Mobility Group Protein B1, a DNA-Binding Structural Chromosomal Protein, as a Novel Substrate of RPE Glutaredoxin

G Hoppe, KE Talcott, Sanjoy K Bhattacharya, John W Crabb, Jonathan E Sears

Purpose: Protein sulfhydryls are reversibly modified by glutathione in response to oxidative stress, known as protein S-glutathionylation. Glutathione/disulfide exchange is catalyzed by the oxidoreductase glutaredoxin. To identify nuclear proteins that undergo redox-dependent S-glutathionylation and enzymatic de-glutathionylation in the retinal pigment epithelium (RPE), we pursued the identification of substrates of glutaredoxin.

Methods: Selective oxidation of sulfhydryls was induced in cultured RPE cell lines (RPE-J or ARPE-19) by 5·10⁻⁴ M diamide. Nuclear proteins were isolated, treated with 2·10⁻⁶ M recombinant glutaredoxin, labeled with biotin-maleimide, purified, and identified by mass spectrometry. Sulfhydryl derivatization by mPEG-maleimide was used to determine the redox status of cysteine residues. Cellular localization and mobility of EGFP-fusion proteins were monitored using live fluorescent microscopy and fluorescence recovery after photobleaching (FRAP). Cysteine mutants were generated by PCR-based mutagenesis.

Results: High mobility group protein B1 (Hmgb1), a DNA-binding structural chromosomal protein and transcriptional co-activator was identified as a substrate of glutaredoxin. Hmgb1 contains 3 cysteines, Cys23, 45, and 106 and their irreversible alkylation reduces Hmgb1 DNA binding. After oxidative stress, Cys23 and Cys45 readily form intramolecular disulfide bridge, whereas Cys106 remains in the reduced form. EGFP-tagged wild type Hmgb1 and endogenous Hmgb1 co-localized in the nucleus. Replacement of Cys23 and/or 45 with serines did not affect the nuclear distribution of the mutant proteins, while C106S and triple cysteine mutations impaired nuclear localization of Hmgb1. Intracellular mobility of Hmgb1 was severely impaired by the mutation of Cys106, but not of Cys23 or 45.

Conclusions: Hmgb1 is a member of a large group of HMG-box containing transcription factors and co-activators, which include regulators of retinal development and survival. Hmgb1 and its homologue Hmgb2 are the only HMG-box proteins that contain cysteines which may be targets of reversible protein S-glutathionylation. Cys23 and 45 appear to confer redox-sensitivity to Hmgb1 by inducing conformational and functional changes in response to oxidative stress. Cys106 appears to govern the nucleocytoplasmic shuttling of Hmgb1.
Insulin Receptor Substrate 2 is Essential for Maturation and Survival of Photoreceptor Cells

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Insulin receptor substrates (Irs-proteins) integrate signals from the insulin and insulin-like growth factor-1 (IGF1) receptors with other processes to control cellular growth, function, and survival. Here, we show that Irs2 promoted the maturation and survival of photoreceptors in the murine retina immediately after birth. Irs2 was mainly localized to the outer plexiform layer as well as to photoreceptor inner segments. It was also seen in ganglion cells and inner plexiform layer but in smaller amounts. Compared with control littermates, Irs2 knock-out mice lose 10% of their photoreceptors 1 week after birth and up to 50% by 2 weeks of age as a result of increased apoptosis. The surviving photoreceptor cells developed short organized segments, which displayed proportionally diminished but otherwise normal electrical function. However, IGF1-stimulated Akt phosphorylation was barely detected, and cleaved/activated caspase-3 was significantly elevated in isolated retinas of Irs2-/- mice. When diabetes was prevented, which allowed the Irs2-/- mice to survive for 2 years, most photoreceptor cells were lost by 16 months of age. Because apoptosis is the final common pathway in photoreceptor degeneration, pharmacological strategies that increase Irs2 expression or function in photoreceptor cells could be a general treatment for blinding diseases such as retinitis pigmentosa.
Management of Subretinal Macular Haemorrhage by Direct Administration of Tissue Plasminogen Activator

RP Singh, C Patel, Jonathan E Sears

**Background/Aims:** Recent studies on the treatment of acute subretinal macular haemorrhage have shown that the volume of the clot and the time to evacuation have strong prognostic factors for visual outcome. A novel technique for surgical evacuation of these lesions involves direct injection of tissue plasminogen activator (t-PA) into the haematoma using pars plana vitrectomy. The aim of this study was to evaluate the clinical outcomes of this recently described procedure.

**Methods:** Seventeen consecutive patients with subretinal macular haemorrhages caused by age related macular degeneration were enrolled. Patient demographics, acuities, and fluorescein angiograms were obtained for all evaluations. All patients underwent complete three port pars plana vitrectomy to enable direct cannulation of the subretinal space and injection of 48 mug of t-PA, partial fluid-air exchange, 1 hour face up supine positioning postoperatively, followed by upright positioning overnight.

**Results:** 88% of patients within the study had stabilisation or improvement of visual acuity. Nine patients had total clearing of the macular haemorrhage and eight patients had subtotal clearing. Two patients had recurrence of the haemorrhage after the procedure and one patient underwent repair for retinal detachment. Occult lesions demonstrated similar outcomes to classic or predominately classic lesions. Nine patients required no therapy after the study to treat subfoveal neovascularisation.

**Conclusions:** This study represents one of the largest case series to date showing that direct injection of subretinal t-PA with air-fluid exchange only and no intraoperative clot lysis period can have favourable results.
Microsomal Glutathione S-Transferase 1 in the Retinal Pigment Epithelium: Protection against Oxidative Stress and a Potential Role in Aging

A Maeda, John W Crabb, K Palczewski

High oxygen tension, exposure to light, and the biochemical events of vision generate significant oxidative stress in the retina and the retinal pigment epithelium (RPE). Understanding the mechanisms and basis of susceptibility to progressive retinal diseases involving oxidative damage such as age-related macular degeneration (AMD) remains a major challenge. Here microsomal glutathione S-transferase (MGST1) is shown to be a dominant, highly expressed enzyme in bovine and mouse RPE microsomes that displays significant reduction activity toward synthetic peroxides, oxidized RPE lipids, and oxidized retinoids. This enzymatic reduction activity (GPx) can be partially neutralized with a monoclonal anti-MGST1 antibody developed in this study. MGST1-transfected HEK293 cells exhibited greater viability (70 ± 4% survival) compared with untransfected control cells (46 ± 4% survival) when challenged with 20 µM H2O2, and greater viability of MGST1-transfected cells following challenge with oxidized docosahexaenoic acid was also observed. Cultured ARPE19 cells transfected with silencing MGST1 siRNAs exhibited lower expression of MGST1 (12% and 26% of the controls) and significantly lower GPx activity (44 ± 13%) and, thus, were more susceptible to oxidative damage. Immunoblotting revealed that the in vivo expression of MGST1 in mouse RPE decreases 3-4-fold with age, to trace levels in 18-month-old mice. GPx activity in the RPE was also found to be reduced in 12-month-old mice to ~67%. These results support an important protective function for MGST1 against oxidative insult in the RPE that decreases with age and suggest that this enzyme may play a role in the development of age-related diseases such as AMD.
Microsphere Luminex Technology for the Analysis of Microliters of Vitreous Fluid
Sophie J Bakri, Tracey Bonfield, Peter K Kaiser, Victor L Perez

**Purpose**: Pilot study to determine the feasibility of analyzing microliters of vitreous fluid using microsphere luminex technology. Microsphere Luminex technology allows simultaneous analysis of 100 samples as small as 50 microliters for various cytokines and biochemical factors. It has been previously used for analysis of other bodily fluids but its application for vitreous analysis has not been described.

**Methods**: Vitreous samples were obtained from 6 patients during scheduled pars plana vitrectomy. Diagnoses were epiretinal membrane (2), posterior hyaloidal traction in a diabetic patient (1), retained lens material after cataract extraction (1), macular hole (1) and vitreous hemorrhage due to choroidal neovascularization from age-related macular degeneration. The vitreous samples were analyzed neat, diluted with phosphate buffered saline (PBS) and pretreated with testicular hyaluronidase to determine both the effect of dilution on the sample and whether the hyaluronidase digestion was necessary to reduce the viscosity of the sample in the machine. For the control group, a standard curve for PBS with and without HA was performed. Microsphere Luminex technology was used with a 22-plex kit, which analyzed the following 22 cytokines: IL-1β, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-12, IL-13, IL-15, IL-17, IFN-γ, TNF-α, GMCSF, IL-1α, Eotaxin, G-CSF, IP-10, MCP-1, MIP-1α and RANTES.

**Results**: In these samples, the most dominant cytokines were IL-8, IP-10 and MCP-1. The patient with vitreous hemorrhage had the highest levels of cytokines. Pretreatment with hyaluronidase was not necessary for the vitreous analysis and produced different results in the control group.

**Conclusion**: This pilot study shows that Microsphere Luminex technology is feasible for vitreous analysis. Pretreatment with hyaluronidase was not necessary for the vitreous analysis.
Microvilli Defects in Retinas of Ezrin Knockout Mice

Vera L Bonilha, Mary E Rayborn, I Saotome, Al McClatchey, Joe G Hollyfield

Ezrin, a member of the ezrin/moesin/radixin (ERM) family, localizes to microvilli of epithelia in vivo, where it functions as a bridge between actin filaments and plasma membrane proteins. In the eye, ezrin has been localized to both apical microvilli of Muller cells and retinal pigment epithelium (RPE) apical microvilli and basal infoldings. In the present study, we analyze these structures in the eyes of early postnatal ezrin knockout mice. This analysis indicates that the loss of ezrin leads to substantial reductions in the apical microvilli and basal infoldings in RPE cells and in the Muller cell apical microvilli. The absence of apical microvilli in the RPE is accompanied by the presence of microvilli-like inclusions (MIs) in the RPE cytoplasm. Finally, photoreceptors in the ezrin knockout animals show substantial retardation in development as compared to their wild type littermates.
Mutation Screen in the CLUL1 Gene in 380 Patients with Age-Related Macular Degeneration

G Sturgill, GJ Pauer, E Simpson, E Bala, S Yaniglos, John W Crabb, Joe G Hollyfield, Hilel Lewis, Neal S Peachey, Stephanie A Hagstrom

Purpose: Clusterin (CLU) is expressed in many tissues and appears to function in a wide range of chaperone processes including membrane recycling and neurodegeneration. A retina-specific clusterin-like protein (CLUL1) was recently shown to be expressed in cone photoreceptor cells. Since CLUL1 is a common drusen protein in age-related macular degeneration (AMD) eyes, we investigated CLUL1 as a candidate gene for AMD.

Methods: All eight coding exons have been screened for mutations in 380 unrelated patients with AMD using exon-by-exon SSCP. Variant bands detected by SSCP were further analyzed by direct genomic sequencing.

Results: Two sequence changes (IVS2+18C→T and IVS8+10insTGCCTGGT TAGGAA) were identified in CLUL1. The IVS2+18C→T change had an allele frequency of 49.6% in patients and 42.8% in age-matched normal controls. IVS8+10(14-bp ins) was identified heterozygously in one patient with AMD and two normal controls, all of African American decent. The minor allele frequency for this sequence change is 0.2% in patients and 0.6% in normals.

Conclusions: We report two sequence changes in CLUL1 in patients with AMD; however, we were unable to associate these changes with disease. Both changes are likely nonpathogenic polymorphisms. Our data suggests that mutations in this gene are rare. It is possible that pathogenic mutations in this gene cause a form of retinal degeneration not included in our patient set.
OCT and Angiographic Analysis of Neovascular AMD Treated With PDT in Combination With Intravitreal Triamcinolone Acetonide vs. PDT Alone

R Ufret-Vincenty, DR Williams, Peter K Kaiser

**Purpose:** To evaluate optical coherence tomography (OCT) and fluorescein angiographic (FA) findings in choroidal neovascularization.

**Methods:** Retrospective review of FA and OCT for lesion type/size, foveal thickness, cystoid macular edema (CME) index, maximal retinal thickness, subretinal fluid (SRF), and pigment epithelial detachment (PED).

**Results:** 34 eyes evaluated were predominantly classic (PC, 10 eyes), minimally classic (MC, 13 eyes), and occult only (OC, 11 eyes). Foveal thickness and CME index were significantly greater in MC (P<0.05). SRF and PED thickness were significantly greater in OC (P<0.05).

**Conclusion:** Different angiographic lesions have characteristic features on OCT.
Ocular Abnormalities in Large\textsuperscript{myd} and Large\textsuperscript{vls} Mice, Spontaneous Models for Muscle, Eye, Brain Diseases

Y Lee, S Kameya, GA Cox, J Hsu, W Hicks, TP Maddatu, RS Smith, JK Naggert, Neal S Peachey, PM Nishina

Mutant genes, fukutin, \textit{POMGNT1} and \textit{POMT1} that cause diseases characterized by muscle, eye, and brain dysfunction have been predicted to function in the process of glycosylation (Grewal et al., 2001; Hayashi et al., 2001; Kano et al., 2002; Beltran-Valero De Bernabe et al., 2002). Yet in the mouse model, myodystrophy (\textit{myd}) in which \textit{Large}, a putative glycosyltransferase, is mutated, previous reports indicate no gross ocular histopathological findings (Michele et al., 2002; Holzfeind et al., 2002). Here we demonstrate clinical, functional and morphological ocular alterations in mice homozygous for a new allele of the \textit{Large} gene, veils, and for \textit{Large}\textsuperscript{myd} mice. Clinically, vitreal fibroplasia, abnormal retinal vessels, and fluorescein leakage from the retinal vasculature were observed in mice homozygous for \textit{Large}\textsuperscript{veils} or \textit{Large}\textsuperscript{myd}. These vascular defects associated with \textit{Large} mutations may, in part, be due to the extreme disorganization of the astrocytic template on which endothelial cells migrate in the retina. Abnormal electroretinograms recorded from \textit{Large}\textsuperscript{veils} or \textit{Large}\textsuperscript{myd} mice were accompanied by disorganization of the outer plexiform layer with bare synaptic ribbons and a dramatic reduction in the number of synaptic complexes. Additionally, disruption of the internal limiting membrane with ectopic amacrine and/or ganglion cells were observed in the vitreous of \textit{Large}\textsuperscript{veils} and \textit{Large}\textsuperscript{myd}-mice. Interestingly, while all components of the dystrophin glycoprotein complex appeared to be present in the outer plexiform layer, albeit at reduced levels, they are absent in the internal limiting membrane of the retina of affected mice. Finally, hypoglycosylation of $\alpha$-dystroglycan previously implicated in muscle and brain defects, is also observed in the retina and may contribute to the ocular abnormalities in \textit{Large}\textsuperscript{veils} and \textit{Large}\textsuperscript{myd}. 
Optic Nerve Fractionation for Proteomic Analysis

Xiarong Gu, John S Crabb, SP Annangudi, Vera L Bonilha, Karen G Shadrach, Joe G Hollyfield, Sanjoy K Bhattacharya, John W Crabb

Purpose: To evaluate the efficacy of solution state isoelectricfocusing (IEF) and 2D polyacrylamide gel electrophoresis (2D PAGE) for fractionation of optic nerve for proteomic analysis.

Methods: Optic nerve was dissected from normal human eyes obtained through the Cleveland Eye Bank and the Eye Donor Program of the Foundation Fighting Blindness, Inc. Optic nerve protein extraction efficiency was evaluated in 7M urea and 2M thiourea containing either 6% C7BzO, 3% ASB-14, 3% dodecyl-maltoside (DM) or a proprietary detergent solution. Soluble protein was quantified by the Bradford and bicinchoninic assays. Following solution state IEF using the Multicompartment Electrolyzer (MCE, Proteome Systems) and 2D PAGE, gel spots were excised, digested in situ with trypsin and proteins identified by capillary LC MS/MS.

Results: Optic nerve extracts from the four detergents produced similar 1D SDS-PAGE profiles, however, ASB-14 and dodecylmaltoside yielded slightly more soluble protein. Solution state IEF in 3% DM separated optic nerve proteins into pH fractions 3-5, 5-6.5, 6.5-8, and 8-11. After IEF, 2D PAGE demonstrated distinctly different patterns for each pH fraction. A total of 153 optic nerve proteins were identified by mass spectrometric analysis of select 2D gel spots. For most of these proteins, the sequence calculated pH was in good agreement with the pH range of the trapping chamber from which it was found.

Conclusions: Optic nerve contains significant lipid-rich myelin membranes and constitutes one of the more difficult tissues from which to extract and analyze soluble protein. Solution state IEF followed by 2D gel analyses have provided a productive approach for fractionating milligram amounts of optic nerve protein. The proteins identified in this study represent the most extensive catalogue to-date of human optic nerve proteins.
Pharmacological Studies of the Mouse Cone Electroretinogram

S Sharma, Sherry L Ball, Neal S Peachey

Electroretinography provides a useful noninvasive approach to evaluate cone pathway activity. Despite wide application of the cone ERG to characterize retinal function in transgenic mice and mouse models of human hereditary retinal disease, the cellular origins of the mouse cone ERG have not been well defined. Here, we address this issue using a pharmacological approach that has been previously applied to other species. Agents that block receptor activation at well-defined retinal loci were dissolved in saline and injected into the vitreous of anesthetized adult BALBc/ByJ mice; cone ERGs were recorded 1-2 hours later. Analysis of the resulting waveforms indicated that the mouse cone ERG includes a cornea-negative component that is derived from the activity of cone photoreceptors and retinal glial (Müller) cells. Similar to other species, activity of cone depolarizing bipolar cells contributes a large amplitude cornea-positive potential to the mouse cone ERG. In contrast to primate but similar to rat, the mouse cone ERG includes only a small contribution from hyperpolarizing bipolar cell activity. The inner retina appears to contribute to both the a- and b-waves of the mouse cone ERG. These results provide a foundation for interpreting changes in the waveform of the mouse cone ERG that may be observed following genetic alteration or other experimental treatment.
Plexiform Pigmented Schwannoma of the Uvea

E Saavedra, Arun D Singh, Jonathan E Sears, NB Ratliff

Schwannoma is a slow growing solitary tumor that preferentially involves spinal nerve roots, and sympathetic, cervical, and vagus nerves. There are several clinico-pathologic variants of schwannoma, including schwannoma with a degenerative change (ancient schwannoma), cellular schwannoma, plexiform schwannoma, epithelioid schwannoma, and melanotic schwannoma. About 10% of cases of schwannomas are associated with multi-system disorders such as neurofibromatosis, schwannomatosis, multiple meningiomas, and Carney complex. Schwannoma rarely present as an intraocular tumor and is often mis-diagnosed as malignant melanoma. Immunohistochemical positivity with S-100 stain and demonstration of long-spaced collagen (Luse bodies) are helpful in establishing the diagnosis. In this article, we review the clinical and histopathological findings of a sporadic plexiform pigmented schwannoma involving the iris, ciliary body, and the choroid.
Presumed Sterile Endophthalmitis Following Intravitreal Triamcinolone Acetonide Injection


Background and objective: To report acute postoperative, presumed sterile endophthalmitis following intravitreal injection of triamcinolone acetonide (IVTA).

Patients and methods: Retrospective, interventional, multicenter study of patients with acute sterile endophthalmitis following IVTA injection.

Results: A total of 922 IVTA injections were performed. Eight eyes of 8 patients with presumed sterile endophthalmitis were identified. The incidence of endophthalmitis was 0.87% (95% confidence interval, 0.38% to 1.70%). Median time to presentation was 1.5 days (range, 1 to 7 days). Median presenting visual acuity was 20/563 (range, 20/80 to light perception). Initial treatment included vitreous tap and injection of antibiotics (n = 4), pars plana vitrectomy and injection of intravitreal antibiotics (n = 2), or systemic treatment alone with oral levofloxacin (n = 2). Six of 6 intraocular cultures were sterile. Median follow-up was 5.9 months (range, 4 to 9 months) with a median visual acuity at last follow-up of 20/75 (range, 20/40 to counting fingers).

Conclusions: Acute presumed sterile endophthalmitis following IVTA injection presents early in the postoperative period. Visual outcomes are generally good.
Protection Against Light-Induced Retinal Degeneration

DT Organisciak, RM Darrow, L Barsalou, John W Crabb

Intense visible light exposure in vivo leads to the synchronous involvement and subsequent loss of numerous photoreceptors. The process is triggered by extensive and/or prolonged rhodopsin bleaching and results in cell death via an apoptotic mechanism. Compelling evidence implicates oxidative stress as an early and key component, as a variety of antioxidants prevent light damage. Retinal light damage also exhibits a circadian dependence with damage greatest for exposures beginning at 1am and least for exposures at 5pm. The lack of damage at 5pm is attributed to the expression of endogenous factors which appear to include retinal crystallins. We used RT-PCR to examine retinal gene expression in rats treated with the synthetic antioxidant dimethylthiourea (DMTU), before or after the onset of light, and 2D gel electrophoresis/western/MS analysis to study protein expression before and after light exposure. Intense light exposure leads to a rapid induction of transcription factors thought to be involved in light damage, including members of the c-fos and c-Jun families. DMTU pretreatment completely prevents photoreceptor loss and decreases transcription factor expression, but fails to do so when given 1 hr after the onset of light. Intense light also results in carboxyethyl pyrrole protein adducts, formed from the oxidation of docosahexaenoic acid and to other post translational modifications of retinal proteins. The levels of ROS crystallins change over the course of day and night and move into and out of ROS during hyperthermia suggesting a protective role for this class of small heat shock proteins. Our data indicates light damage is triggered rapidly by rhodopsin bleaching and that exogenous antioxidants or endogenous factors need to be present at the start of light exposure to prevent damage and photoreceptor cell degeneration.
Protein Expression Patterns of Cultured Human RPE Cells Under Hyperglycaemic Condition Investigated by Proteome Analysis

T Yokoyama, K Yamane, A Minamoto, HK Mishima, H Yamashita, G Hoppe, Jonathan E Sears

Purpose: To identify differential protein expression patterns of retinal pigment epithelial (RPE) cells exposed to increased glucose concentrations equivalent to acute hyperglycemia in the diabetic patient.

Methods: Human RPE cells (ARPE-19) were cultured for 4 days with normal blood glucose concentration (5.5 mM D-glucose), followed by exposure to either normal (5.5 mM) or high (33 mM) concentrations of D-glucose for 48 hours. Protein extracts of glucose-treated RPE cells were then subjected to comparative proteome analysis based on 2-D gel electrophoresis. Protein spots were visualized by silver staining. The differentially expressed proteins were excised and digested in-gel with trypsin, then analyzed by matrix-assisted laser desorption/ionisation time-of-flight (MALDI-TOF) mass spectrometry.

Results: The expression levels of cathepsin B, glutathione peroxidase and heat shock protein 27 were increased, and that of protein disulfide isomerase decreased in high glucose treated RPE compared to normal glucose. The isoelectric point of copper/zinc-containing superoxide dismutase shifted toward acidic region in response to high glucose.

Conclusions: Systematic survey of protein expression has revealed that RPE cells respond to acute, pathologically high glucose levels by the elevated expression of anti-oxidant and proteolytic enzymes.
Proteomic Analysis of Vitreous from Diabetic Macular Edema

M Ouchi, K West, John W Crabb, S Kinoshita, M Kamei

To identify and analyze diabetic macular edema (DME)-related proteins in the vitreous, en masse, using two-dimensional gel (2D gel) electrophoresis and mass-spectrometry (MS). Vitreous samples were collected from 20 eyes with pre-proliferative diabetic retinopathy associated with DME (DME group) and without DME (non-DME group). They were subjected to 2D gel electrophoresis and spot intensities were compared between groups. Apparently visible spots were excised from the gel, and the proteins were identified by liquid chromatography tandem MS (LC MS/MS) sequence analysis. We identified 14 proteins from the DME group, and 15 proteins from the non-DME group. The intensity of 8 spots was markedly higher in DME than non-DME samples and one spot was detected only in non-DME samples. From the eight spots, six proteins were identified, including PEDF, Apo A-4, ApoA-1, Trip-11, PRBP, and VDBP. On the other hand, Apo H was expressed only in non-DME. Certain vitreous expressed exclusively in DME and lacked in DME. These chemical mediators in the posterior vitreous may play a role in the pathogenesis of DME.
Proteomic and Ultrastructural Analyses of Human Lipofuscin

BG Gugiu, M Rozanowska, B Rozanowski, Mary E Rayborn, Vera L Bonilha, Xiarong Gu, RG Salomon, Joe G Hollyfield, ME Boulton, John W Crabb

Purpose: The progressive accumulation of lipofuscin in the retinal pigment epithelium (RPE) correlates with the pathogenesis of age-related macular degeneration (AMD). We seek a better molecular understanding of the sources and consequences of lipofuscin accumulation, including the protein content of lipofuscin.

Methods: Human RPE lipofuscin was purified by conventional sucrose density gradient centrifugation methods. Lipofuscin granule purity was evaluated by light, fluorescence, confocal, and electron microscopy. Lipofuscin preparations were extracted with chloroform/methanol then the chloroform insoluble material was extracted with SDS and subjected to SDS-PAGE, gel bands excised and proteins identified by LC MS/MS. Western analysis was used to probe for oxidative protein modifications.

Results: Ultrastructural analyses of lipofuscin purified by conventional methods revealed a heterogeneous core structure composed of lipofuscin granules surrounded by substantial extra-granular material. The chloroform insoluble lipofuscin fraction of the conventional preparation exhibited many fuzzy Coomassie blue stained SDS-PAGE bands, suggesting post-translational modifications. Western blot analysis confirmed the presence of abundant carboxyethylpyrrole adducts. Over 160 proteins were identified, ~33% of which exhibited apparent mass additions. Essentially “pure” lipofuscin granules, free of extra-granular material, were obtained by proteolytic digestion of the conventional preparation. Boiling the purified granules in SDS has so far failed to yield SDS-PAGE detectable bands with Coomassie or silver staining.

Conclusions: Lipofuscin granules appear to be embedded in a protein “matrix” similar in content to drusen. Proteomic characterization of purified lipofuscin granules is under way.
Results of NICU Exams for Retinopathy of Prematurity at the Cleveland Clinic Foundation

C Sonnie, Jonathan E Sears

Purpose: To evaluate the physical findings of ROP exams and treatment outcomes during a 24 month period at the Cleveland Clinic NICU to compile a database as a resource for ROP investigations.

Methods: Exams were performed on children between 5-7 weeks of life for any infant less than 1500 grams birth weight or 32 weeks gestational age or unstable clinical course or 24 hours of oxygen therapy. A retrospective chart review of exams was conducted to analyse gestational age, birth weight, Zone, Stage, threshold disease, treatment and final outcome.

Results: Total exams were 521 with new exams numbering 240 (2.2 exams per patient). Average birth weight was 750 grams (range 400-1700 grams). Average gestational age was 25 weeks (range 23-32 weeks). Fifty percent of eyes developed ROP, 18.3% of those eyes developed threshold disease (9.1% overall) and were treated with diode laser indirect ophthalmoscopy. One eye (1/44 eyes, 2.3%) progressed to stage IVb detachment after treatment. No infant developed bilateral unfavorable outcome. Zone I eyes were treated with laser if 4/4 plus disease was noted with any ROP. Zone 2 eyes were treated at standard threshold disease. All threshold infants were average 24 weeks gestational age and 503 grams birth weight. Average corrected gestational age at time of treatment was 34 weeks. Thirty-three percent of threshold eyes were Zone I.

Conclusions: Zone I disease comprises an increasing percentage of threshold ROP. Our data suggests that the rate of unfavorable outcomes can be reduced to less than 3 % in level III nursery if Zone I eyes are treated when 4/4 plus disease with any ROP is noted.
Retinal Ganglion Cell Function is Altered in Two Mouse Models of Congenital Stationary Night Blindness

KA Vessey, BT Sagdullaev, B Chang, Neal S Peachey, RG Gregg, MA McCall

X-linked, congenital stationary night blindness (CSNB) types 1 and 2 are caused by mutations in nyctalopin and the $\alpha_{1F}$ subunit of voltage gated calcium channels respectively. The nob1 and nob2 mouse mutants are models for these human diseases and display altered synaptic transmission in the outer retina. We explored overall retinal function in these mutants, and in a transgenic nob1 “rescue” line where nyctalopin expression had been restored in bipolar cells. We used the electroretinogram (ERG) and in vivo single unit, extracellular recording of retinal ganglion cells (RGCs) in light adapted animals. The ERG from both the nob1 and nob2 mice show a decrease in the amplitude of the ERG b-wave compared to wild type (WT) mice. Extracellular RGC recordings in these mutants showed that receptive field organization was similar across WT, nob1 rescue and nob2 mice. In contrast, RGCs in nob1 mice showed no evidence of center surround organization. RGCs of nob1 had significantly reduced sensitivity to light and also displayed an abnormal, rhythmic bursting activity. In nob1 rescues, light sensitivity was restored and was indistinguishable from WT cells. Thus, expression of nyctalopin in bipolar cells restores normal visual function. In nob2 RGCs differences were observed between ON and OFF center cells. OFF center cells were not different from WT in either their light sensitivity or spontaneous activity, while ON center cells had increased light sensitivity and decreased spontaneous activity, resulting in a decreased dynamic range. In general, these data show that nob2 mice have a milder visual deficit than nob1 mice, which is consistent with human CSNB phenotypes.
RPGR ORF15 Isoform Co-Localises with RPGRIP1 at Centrioles and Basal Bodies and Interacts with Nucleophosmin

X Shu, AM Fry, B Tulloch, FDC Manson, A Faragher, A Lennon, P Trojan, A Giessl, U Wolfrum, R Vervoort, John W Crabb, AF Wright

The ORF15 isoform of RPGR (RPGR_{ORF15}) and its interacting protein, RPGRIP1, are mutated in a variety of retinal dystrophies but their functions are poorly understood. The carboxyl terminal (C2) domain of RPGR_{ORF15} (ORF15\textsubscript{C2}) is shown to be highly conserved across 18 mammalian species, suggesting that it is a functionally important domain. We show that this domain interacts with the 40 kD shuttling protein nucleophosmin (NPM) and that both proteins co-localise at centrosomes during metaphase in cultured mammalian cells. At high resolution, both RPGR_{ORF15} and RPGRIP1 co-localise with centriolar $\gamma$-tubulin at all stages of the cell cycle. These localisations were resistant to the microtubule destabilising drug nocodazole. The interaction with NPM was identified by MALDI-ToF mass spectrometry following protein pull-downs with ORF15\textsubscript{C2}. The RPGR_{ORF15}-NPM interaction was confirmed by (i) yeast two-hybrid analyses; (ii) binding of both recombinant and native HeLa cell NPM to RPGR_{ORF15} fusion proteins in vitro; (iii) co-immunoprecipitation of native NPM, RPGR_{ORF15} and RPGRIP1 in bovine retinal extracts; (iv) co-immunoprecipitation of native HeLa cell NPM and transfected RPGR_{ORF15} in cultured cells. NPM is a multifunctional protein chaperone that shuttles between nucleoli and centrosomes and has been associated with licensing of centrosomal division. It is proposed RPGR and RPGRIP1 regulate the docking of NPM and other centrosomal proteins at the centrioles in dividing cells and at basal bodies in photoreceptors. RPGR and RPGRIP join a growing number of centrosomal proteins involved in human disease.
Safety of Anecortave Acetate Suspension by Posterior Juxtascleral Depot in Patients with Exudative Age-Related Macular Degeneration

Peter K Kaiser, D D’Amico, C Beasley

**Purpose:** Review of the safety of the angiostatic cortisene, Anecortave Acetate Suspension and Posterior Juxtascleral Depot (PJD) administration in patients with age-related macular degeneration (AMD).

**Methods:** Detailed ophthalmic examinations including assessments of intraocular pressure and cataractous lens changes as well as periodic physical examinations have been used to establish the clinical safety profile of Anecortave Acetate and the PJD procedure.

**Results:** The clinical development of Anecortave Acetate for the treatment of choroidal neovascular AMD encompasses 883 patients in 8 clinical studies. More than 2000 PJD administrations of Anecortave Acetate suspension or vehicle have been performed. Safety assessments of drug and PJD procedure have been reviewed by an Independent Safety Committee through analysis of ocular (visual acuity, ocular signs, lens opacities, lens abnormalities, eyelid/pupil responsiveness, IOP, posterior segment abnormalities and ocular motility) and non-ocular (general physical examination, EKG, cardiovascular and laboratory) parameters. To date, no drug-related concerns have been raised for ocular or systemic safety. Additionally, PJD administration has been demonstrated as an effective and safe procedure for retinal drug delivery in AMD.

**Conclusion:** Anecortave Acetate and the PJD procedure are safe and well-tolerated by AMD patients when administered every six months for the treatment of age-related macular degeneration.
Similarities in Plasma Oxidative Modifications from Retinal Light Damage and AMD

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Purpose: To identify similarities in pathogenesis between age-related macular degeneration (AMD) and retinal light damage and to identify biomarkers for AMD, we measured plasma carboxyethylpyrrole (CEP) and ethylpyrrole (EP) adducts and autoantibodies. CEP and EP adducts are generated from the oxidation of docosahexaenoate (DHA)-containing lipids.

Methods: Blood was collected from clinically documented AMD and normal, healthy donors at the Cleveland Clinic Foundation and Louis Stokes VA Medical Center. Dark adapted albino rats with or without pretreatment with dimethyl thiourea (DMTU) were exposed to intense green light (1500 lux) for 0, 2, 4, or 8h then sacrificed. Blood was drawn immediately, antioxidants added and plasma prepared. Plasma CEP and EP immunoreactivities and autoantibody titers were measured by ELISA.

Results: Rat plasma CEP immunoreactivity and autoantibody titer increased with light exposure and after 8h were ~1.6x and ~2.8x higher, respectively, without DMTU (n = 6 per time point). With DMTU, CEP values were lower. Rat plasma EP values did not change significantly with light exposure. However, human EP immunoreactivity was ~1.4x greater in AMD plasma (n = 51) relative to normal donors (n = 22). Statistically significant elevation of EP immunoreactivity was observed in human plasma from AREDS AMD category 2 (n = 20), but not in AMD category 3 (n = 17) or AMD category 4 (n = 14).

Conclusions: Elevated CEP immunoreactivity and autoantibody titer in plasma from both light damaged rats and AMD donors support DHA oxidation as a common mechanism of pathogenesis. The lack of elevated EP in light damaged rat plasma and late stage AMD may reflect further modification of EP due to more acute damage. Nevertheless, plasma EP may have utility as an AMD biomarker.
Status of the Feline Retina after Five Years of Subretinal Implantation with an Artificial Silicon Retina

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**Purpose:** Retinal prosthetics are designed to restore vision to patients with photoreceptor degeneration, like retinitis pigmentosa, by contacting the retina to directly stimulate the neural retina. Permanently implanted devices need to have long-term biocompatibility and durability to be a viable treatment for retinal disease. We have previously demonstrated biocompatibility of the subretinal Artificial Silicon Retina (ASR™) device for up to 27 months in the normal feline retina (Pardue et al. 2001. Exp. Eye Res. 73,333-334). Here we describe results obtained in longer follow-up periods.

**Methods:** Normal cats were implanted with ASR™ devices backed with either an iridium oxide or a platinum electrode. Retinal function and implant activity was monitored by periodic ERG recordings. After three (n=3) or five (n=2) years follow-up, the eyes were enucleated and the retinas examined for changes in gross morphology as well as immunohistochemical labeling patterns of 3 primary neurotransmitters of the inner retina: glycine, GABA, and glutamate.

**Results:** The continued presence of an implant spike during ERG recordings indicated that all implants functioned throughout the study period. The ~20% decline in ERG amplitude that was previously noted to accompany surgery and the loss of photoreceptors directly overlying the implant was maintained and did not appear to progress with time. In areas adjacent and distant from the implant, retinas retained normal laminar structure with no signs of inflammation or glial reaction. Directly over the implant, there was loss of photoreceptor nuclei and remodeling of the inner retinal layers, including alterations in the distribution of neurotransmitter labels.

**Conclusions:** The morphological changes above the implant appeared to be caused by the presence of the solid device in the subretinal space possibly related to the blockage of choroidal nourishment. The maintenance of normal function and structure in all other retinal areas indicate that the subretinal ASR™ device is well-tolerated by the retina and is functional in vivo for as long as five years.
The Retinal Pigment Epithelium
Apical Microvilli and Retinal Function

Vera L Bonilha, Mary E Rayborn, Sanjoy K Bhattacharya, Xiaorong Gu, John S Crabb, John W Crabb, Joe G Hollyfield

The RPE performs highly specialized, unique functions essential for homeostasis of the neural retina. These include phagocytosis of photoreceptors shed outer segments, directional transport of nutrients into and removal of waste products from photoreceptor cells and visual pigment transport and regeneration. All of these functions involve the RPE apical microvilli.

The RPE is a low cuboidal epithelium containing very long sheet-like apical microvilli that project into the interphotoreceptor matrix. The microvilli interact with the tips of the rod and cone photoreceptor outer segments extending from the outer retinal surface. The cone-RPE association is much less studied however, as many as 30-40 microvilli can be associated with a single cone. These vary in length with only a few reaching the outer segment. The RPE apical microvilli ensheath the outer segments of photoreceptor cells, extending for as long as half the outer segment. A single RPE microvillous may completely surround the outer segment or multiple microvilli can encircle each other while surrounding the photoreceptor outer segments. Intracellular organelles are mostly absent from the cone-ensheathing microvilli while they are very abundant in the microvilli ensheathing the rod outer segments.

The RPE basal surface is highly infolded and interacts with the underlying Bruch's membrane, an acellular layer separating the RPE from the choriocapillaris. The polarized organization of the RPE is essential for the vectorial transport of different molecules between the choriocapillaris and the neural retina and vice-versa. A unique characteristic of the RPE is the reversed polarity of select proteins such as the Na,K-ATPase pump, EMMPRIN and the adhesion molecule N-CAM. These proteins are found at the apical surface of the RPE, rather than at the basolateral surface as in other epithelia.
Triamcinolone Acetoneide Decreases HIF-1 Binding to Hypoxia Response Element in Cobalt Stimulated Mueller Cells

Jonathan E Sears, G Hoppe

**Purpose:** To identify the molecular mechanism of steroid-induced resolution of macular edema.

**Methods:** Confluent cultures of human Mueller cells (MIO-M1) were serum-starved for 48 hours followed by treatment with 100 µM CoCl$_2$, 1 µg/ml triamcinolone acetonide (TA), or both. Vascular endothelial growth factor (VEGF) secretion was measured in the supernatants of cultures with respect to time by ELISA. VEGF mRNA was analyzed by reverse transcriptase polymerase chain reaction. The activity of the heterodimer hypoxia inducible factor-1 (HIF-1) which is composed of inducible HIF-1α and constitutive HIF-1β subunits was measured by the relative binding of HIF-1 protein to the hypoxia response element (HRE). HIF-1α protein level was determined using Western blot.

**Results:** TA decreased VEGF secretion by at least 50% in the presence of continuous cobalt stimulus. VEGF mRNA decreased 50-100 fold 6 hours post treatment with TA and cobalt compared to cobalt alone. HIF-1α protein peaked at 6 hours after cobalt treatment and was sustained for the entire 24 hour treatment period. HIF-1α protein level was equal in cobalt and TA/cobalt treated cells and partitioned into nuclear not cytosolic fractions. However, HIF-1 binding to the HRE was decreased by 30% in the presence of TA and cobalt compared to cobalt alone.

**Conclusions:** TA decreases VEGF secretion and VEGF mRNA concentration in cobalt stimulated Mueller cells. No effect is seen on total HIF-1α protein level, but HIF-1 activity is decreased. TA may induce a post-translation modification of HIF-1 or availability of cofactors that does not affect nuclear translocation but does affect DNA binding.
Triamcinolone Acetonide Destabilizes VEGF mRNA in Muller cells under Continuous Cobalt Stimulation

Jonathan E Sears, G Hoppe

Purpose: To identify the molecular mechanism of steroid-induced downregulation of vascular endothelial growth factor (VEGF) synthesis in Muller cells.

Methods: Confluent cultures of human Muller cells (MIO-M1) were treated with 100 microM CoCl(2), 1 microg/mL triamcinolone acetonide (TA), or both. VEGF secretion was measured with respect to time by ELISA. VEGF mRNA quantity and stability were analyzed by reverse transcriptase-polymerase chain reaction. The activity of hypoxia-inducible factor (HIF)-1 was measured by the relative binding of HIF-1 protein to the hypoxia response element (HRE), by gel shift and ELISA. The HIF-1alpha protein level was determined with Western blot.

Results: TA decreased VEGF secretion by at least 50% in the presence of continuous cobalt stimulus. VEGF mRNA decreased 50- to 100-fold 6 hours after treatment with TA and cobalt compared with cobalt alone. VEGF mRNA stability was decreased in cobalt-stimulated, TA-treated cells compared with cobalt alone in cells synchronized by exposure to actinomycin D. HIF-1alpha protein level was sustained for the entire 24-hour treatment period and partitioned into nuclear, not cytosolic, fractions. HIF-1 activity was decreased by 20% to 30% in the presence of TA and cobalt compared with cobalt alone.

Conclusions: TA may decrease VEGF synthesis by nongenomic destabilization of VEGF mRNA in cobalt-stimulated Muller cells. There was little effect on the total HIF-1alpha protein level, HIF-1 partitioning, and HIF-1 activity.
Tubby-like Protein 1 (TULP1) Interacts with F-actin in Photoreceptor Cells

Q Xi, GJ Pauer, AD Marmorstein, John W Crabb, Stephanie A Hagstrom

Purpose: TULP1 is a photoreceptor-specific protein of unknown function that, when mutated, can cause retinitis pigmentosa in humans and photoreceptor degeneration in mice. Toward a better understanding of the role of TULP1 in retinal disease, its subcellular localization was sought and the TULP1 protein binding partners identified.

Methods: Immunocytochemistry and subcellular fractionation were used to determine the localization of TULP1 and actin in COS7 cells and photoreceptor cells. Immunoprecipitation from retinal lysates followed by liquid chromatography tandem mass spectrometry and in vitro binding assays was used to identify TULP1-binding partners. Phospholipid binding assays were performed with a commercially available kit.

Results: TULP1 localizes at or near the plasma membrane and associates with the membranous fraction of COS7 cells, probably through binding phosphorylated phospholipids. In addition, TULP1 partitions to the aqueous phase during Triton X-114 extraction. Immunoprecipitation from retinal lysate identified F-actin as a possible TULP1-binding partner. Co-sedimentation assays further support an interaction between TULP1 and actin. In photoreceptor cells, actin and TULP1 colocalize at the inner segment, connecting cilium, and outer limiting membrane.

Conclusions: TULP1 is a cytoplasmic protein that associates with cellular membranes and the cytoskeleton. TULP1 and actin appear to interact and colocalize in photoreceptor cells of the retina. TULP1 may be involved in actin cytoskeletal functions such as protein trafficking that takes place at or near the plasma membrane from the inner segment through the connecting cilium into the outer segment of photoreceptor cells.
Verteporfin Therapy with Adjunct Triamcinolone Acetonide for Subfoveal CNV Due to AMD

Peter K Kaiser, DS Boyer, RA Mittra, RB Feldman, S Dev

**Purpose:** To examine the visual outcomes of patients receiving verteporfin (Visudyne, Novartis Pharma AG) photodynamic therapy (PDT) with adjunctive intravitreal triamcinolone acetate for the treatment of CNV due to AMD, using data from the Visudyne Patient Registry, an Internet-based, secure database.

**Methods:** Retrospective analysis of patients treated for CNV due to AMD with verteporfin PDT and at least 1 adjunctive intravitreal injection of triamcinolone acetate. Triamcinolone was administered before, on the same day, or several days after verteporfin PDT. Snellen visual acuity and potential complications including intraocular pressure elevations, cataract, endophthalmitis/pseudoendophthalmitis, and retinal tears/detachments were recorded at each visit.

**Results:** 335 patients with CNV due to AMD were enrolled by 6 physicians at 5 clinical centers. At baseline, 63% of patients had predominantly classic CNV; 25% had minimally classic CNV; and 12% had occult with no classic CNV. Mean lesion size (GLD) at baseline was 3140 microns and 89% of patients had subfoveal CNV. For data analysis, patients were divided into 2 groups: better VA (baseline VA 20/200 or better, mean 20/100+2) or poorer VA (baseline VA worse than 20/200-1, mean 20/640+2). Of the 335 patients, 200 had a 180-day VA assessment. By 180 days, patients had received a mean of 2.1 and 1.8 verteporfin PDT treatments in the better VA (n=137) and poorer VA (n=63) groups, respectively, and a mean of 1.0 adjunctive triamcinolone treatment in both groups. Mean change in VA from baseline was -8.6 letters (-1.8 lines) in patients with better VA at baseline, and +10.0 letters (+2.0 lines) in patients with poorer baseline VA. Complications were minimal and transient. Results will be discussed in the context of analyses of patients who received verteporfin PDT alone as previously reported from the Visudyne Patient Registry and from randomized controlled trials of verteporfin PDT.

**Conclusions:** Although retrospective case series cannot substitute for randomized controlled trials, this retrospective analysis suggests a benefit in treating patients with the combination of verteporfin PDT and adjunctive intravitreal triamcinolone acetate. Several randomized clinical trials are under way to better assess the role of this combination treatment.
Purpose: To describe the appearance and prevalence of various morphological patterns of diabetic macular edema demonstrated by optical coherence tomography (OCT).

Methods: A retrospective chart review of all patients with clinically evident diabetic macular edema who underwent OCT evaluation at the Cole Eye Institute between May 1998 and December 2002 was performed. The OCT scans were evaluated for the presence of retinal thickening, cystoid macular edema, hyaloidal traction, subretinal fluid, and traction retinal detachment. In addition, the foveal retinal thickness was measured.

Results: A total of 199 eyes from 144 patients were identified. OCT revealed at least five distinct morphologic subgroups of diabetic macular edema: diffuse retinal thickening (192, 96%), cystoid macular edema (114, 57%), subretinal fluid without posterior hyaloidal traction (21, 11%), posterior hyaloidal traction without traction retinal detachment (28, 14%), and posterior hyaloidal traction with traction retinal detachment (3, 2%). Only diffuse retinal thickening appeared alone, and was seen in 81 (41%) eyes. The other patterns did not appear alone, and occurred in the following combinations: diffuse retinal thickening combined with cystoid macular edema (64, 32%); diffuse retinal thickening, cystoid macular edema, and subretinal fluid (17, 8.5%); diffuse retinal thickening, cystoid macular edema, and posterior hyaloidal traction (16, 8.0%); diffuse retinal thickening and posterior hyaloidal traction (11, 5.5%); diffuse retinal thickening, cystoid macular edema, posterior hyaloidal traction and traction retinal detachment (2, 1%); and diffuse retinal thickening, posterior hyaloidal traction, and traction retinal detachment (1, 0.5%). The mean retinal thickness varied within each subtype: diffuse retinal thickness averaged 411.0 ± 132.9 microns (range 215-772 microns), cystoid macular edema 473.7 ± 126.0 microns (235-772), subretinal fluid without posterior hyaloidal traction 547.1 ± 93.0 microns (376-760), posterior hyaloidal traction without traction retinal detachment 448.9 ± 123.9 microns (375-765), and posterior hyaloidal traction with traction retinal detachment 576.8 ± 124.3 microns (376-759).

Conclusions: Diabetic macular edema exhibits at least five different morphologic patterns on optical coherence tomography with varying incidence rates. All patterns resulted in increased retinal thickness.
Section 10

Uveitis
Comparison of Adverse Events From Intravitreal Triamcinolone in Uveitis and Nonuveitis Patients

Careen Y Lowder, Scott D Smith, Victor L Perez, Sophie J Bakri, Jonathan E Sears, Peter K Kaiser

**Purpose:** To compare intravitreal triamcinolone (IVT) adverse events in uveitis and nonuveitis patients.

**Methods:** Retrospective review of adverse events in 64 patients receiving 4 mg IVT.

**Results:** Uveitis patients experienced a greater IOP rise (11.8 ± 8.5 mm Hg versus 4.1 ± 5.3 mm Hg, P = .007) and posterior subcapsular cataract progression (85.7% versus 32%, P = .03) than nonuveitis patients over 14 months follow-up. Uveitis patients required more frequent interventions for elevated IOP (69.2% versus 21.6%, P = .002), and uveitis remained a significant risk factor for IOP elevation in a multivariate analysis.

**Conclusion:** IOP elevation and cataract progression occurred with greater frequency in uveitis patients receiving IVT.
Differential Effectiveness of Etanercept and Infliximab in Ocular Inflammation

Careen Y Lowder, A Galor, Victor L Perez

**Purpose:** Anti-tumor necrosis factor alpha (anti-TNFα) agents (etanercept and infliximab) are being increasingly used in refractory inflammatory eye diseases. We reviewed our patients on anti-tumor necrosis factor therapy to determine whether these medications are equally efficacious.

**Methods:** Case records of 18 patients treated with anti-TNFα therapy were reviewed for demographic information, ocular and systemic diagnoses, duration and dose of anti-TNFα treatment, concomitant ocular and systemic immunosuppressive medications, and treatment response. Treatment response was designated: “complete success” if at last follow-up, ocular inflammation was in remission on an anti-TNFα therapy, along with systemic and ocular medications; “partial success” if at last follow-up, ocular inflammation was improved from before treatment but was not in complete remission; “failure” if at last follow up patient had no improvement or an increase in ocular inflammation from prior to treatment.

**Results:** Thirteen of 15 patients (87%) treated with infliximab for an average of 15 months (± 14) had either complete (8 patients, 53%) or partial control (5 patients, 33%) of their ocular inflammation. Two patients (13%) failed infliximab treatment. Twelve of 15 patients (80%) decreased topical corticosteroid use. Prednisone was tapered and discontinued in five of seven patients and two patients were maintained on 5 mg daily. In contrast, all seven patients (100%) treated with etanercept for an average of 23 months (± 21) failed treatment. Five of these seven patients were changed to infliximab therapy and four of five (80%) achieved either complete or partial response after initiation of therapy with infliximab. Three of seven patients (43%) initially responded to etanercept. A significant difference was found between patients treated with infliximab compared to those treated with etanercept with regards to response to therapy (p=0.0003) and topical corticosteroid use (p=0.015).

**Conclusions:** Infliximab was more effective than etanercept in the treatment of recalcitrant uveitis. Long term data on efficacy is not yet available and persistent response to treatment remains to be assessed.
Etanercept (Enbrel) Associated Inflammatory Eye Disease: Case Report and Review of the Literature

M Taban, William J Dupps Jr, B Mandell, Victor L Perez

**Purpose:** To report a case of severe anterior uveitis flare following administration of etanercept (Enbrel) for ankylosing spondylitis and to review the literature pertaining to inflammatory eye disease associated with use of etanercept.

**Methods:** Clinical data including medical history were collected in a 52-year old female with chronic symptomatic ankylosing spondylitis. The role of etanercept in the patient’s temporal clinical response, both systemic and ocular, was assessed. A detailed review of literature was conducted in PUBMED using such terms as etanercept, Enbrel, uveitis, scleritis, and inflammatory eye disease. Additional studies were identified from bibliographies of relevant articles and published proceedings.

**Results:** After more than a year of treatment with etanercept, a patient with a history of ankylosing spondylitis and bilateral anterior uveitis exhibited acute exacerbation of uveitis that was temporally related to etanercept injections. Rechallenge was associated temporally with worsening of symptoms, and dechallenge in concert with aggressive systemic treatment resulted in rapid resolution. Seventeen cases of inflammatory eye disease (uveitis, scleritis, myositis) believed to be associated with etanercept were found in the recent literature.

**Conclusions:** Ocular inflammation is a potential adverse event following the use of etanercept both in previously uninvolved eyes and in previously inflamed eyes subjected to rechallenge. Careful surveillance of patients on etanercept is warranted to determine the true risk and associated factors related to their occurrence.
Section 11

Cell Biology
Physical and Functional Interaction of DNA Methyltransferase 3a With Mbd3 and Brg1 in Mouse Lymphosarcoma Cells

J Datta, S Majumder, S Bai, K Ghoshal, H Kutay, DS Smith, John W Crabb, ST Jacob

Dnmt3a and Dnmt3b are de novo DNA methyltransferases that also act as transcriptional repressors independent of methyltransferase activity. To elucidate the underlying mechanism of transcriptional repression, Dnmt3a was purified from mouse lymphosarcoma cells (P1798) by extensive fractionation on five different chromatographic matrices followed by glycerol density gradient centrifugation. Liquid chromatography electrospray tandem mass spectrometry analysis of Dnmt3a-associated polypeptides identified the methyl CpG binding protein Mbd3, histone deacetylase 1(Hdac1), and components of Brg1 complex (Brg1, Baf155, and Baf57) in the purified preparation. Association of Dnmt3a with Mbd3 and Brg1 was confirmed by coimmunoprecipitation and coimmunolocalization studies. Glutathione S-transferase pulldown assay showed that the NH2-terminal ATRX homology domain of Dnmt3a interacts with the methyl CpG binding domain of Mbd3 and with both bromo and ATPase domains of Brg1. Chromatin immunoprecipitation assay revealed that all three proteins are associated with transcriptionally silent methylated metallothionein (MT-I) promoter in the mouse lymphosarcoma cells. To understand the functional significance of their association with the promoter, their role on the MT-I promoter activity was analyzed by transient transfection assay. The results showed that Mbd3 and Dnmt3a specifically inhibited the methylated promoter, and the catalytic activity of Dnmt3a was dispensable for the suppression. In contrast, the wild-type but not the ATPase-inactive mutant of Brg1 suppressed MT-I promoter irrespective of its methylation status, implicating involvement of ATP-dependent chromatin remodeling in the process. Coexpression of two of the three interacting proteins at a time augmented their repressor function. This study shows physical and functional interaction of Dnmt3a with components of nucleosome remodeling machinery.
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Specialty Interests: Vitreoretinal diseases, complicated macular holes, macular surgery, age-related macular degeneration, retinal detachment, diabetic retinopathy

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Specialty Interests: Ocular inflammatory diseases, uveitis and pathology

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Medical School: Wayne State University, Detroit
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Specialty Interests: Pediatric ophthalmology, adult strabismus

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Specialty Interests: Cataract and implant surgery, glaucoma, diabetes

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Specialty Interests: Corneal and external disease, corneal transplantation, adult cataract surgery
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Medical School: University of Pennsylvania School of Medicine, Philadelphia
Specialty Training: Residency – University Hospital, Cincinnati, Ohio
Specialty Interests: Cataract surgery, refractive surgery, glaucoma

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Specialty Training: University of Illinois, Chicago
Research Interests: Hereditary retinal disease

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Specialty Interests: Cornea and external diseases, ocular inflammation and uveitis, ocular immunology, corneal transplantation and anterior segment reconstruction

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Medical School: Columbia University College of Physicians & Surgeons, New York
Specialty Training: Residency – Wills Eye Hospital, Philadelphia; Fellowship – Johns Hopkins Hospital, Wilmer Eye Institute, Baltimore; Fellowship – Jules Stein Eye Institute, University of California School of Medicine, Los Angeles
Specialty Interests: Ophthalmic plastic and reconstructive surgery, cosmetic eyelid and facial surgery, blepharoplasty, laser resurfacing, cosmetic Botox injections
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Medical School: State University of New York School of Medicine and Biomedical Sciences, Buffalo
Specialty Training: Residency – The Cleveland Clinic Foundation, Cleveland, Ohio; Fellowship – Bascom Palmer Eye Institute-Anne Bates Leach Eye Hospital, Miami
Specialty Interests: Adult and pediatric glaucomas, glaucoma filtering surgery, glaucoma implants, mitomycin c, combined glaucoma and cataract surgery

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Medical School: Case Western Reserve University, Cleveland, Ohio
Specialty Training: Residency – Mount Sinai Medical Center, Cleveland, Ohio; Fellowship – Tulane University Hospital & Clinics, New Orleans
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Office: 216.444.7963
Medical School: Johns Hopkins University School of Medicine, Baltimore
Specialty Training: Residency – Wilmer Ophthalmological Institute, Baltimore; Fellowship – Wills Eye Hospital, Philadelphia
Specialty Interests: Medical retina, age-related macular degeneration, diabetic retinopathy
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Medical School: Yale University School of Medicine, New Haven, Connecticut

Specialty Training: Residency – Yale-New Haven Hospital, New Haven, Connecticut; Fellowship – Emory University Hospital, Atlanta

Specialty Interests: Pediatric and adult vitreoretinal surgery, pediatric retinal detachment, inherited vitreoretinal disorders, retinopathy of prematurity and other acquired vitreoretinal diseases, detachment secondary to degenerative disorders such as myopia and macular degeneration

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Medical School: Case Western Reserve University, Cleveland, Ohio

Specialty Training: Residency – University Hospitals of Cleveland, Ohio

Specialty Interests: General ophthalmology, cataract/implant surgery

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Medical School: Jawaharlal Institute of Post Graduate Medical Education and Research, Pondicherry, India

Specialty Training: Residency – University of Florida College of Medicine, Gainesville; Fellowship – Wills Eye Hospital, Philadelphia

Specialty Interests: Retinoblastoma, uveal melanoma, eyelid and conjunctival tumors, von Hippel-Lindau disease
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Medical School: Yale University School of Medicine, New Haven, Connecticut

Specialty Training: Residency – Massachusetts Eye and Ear Infirmary/Harvard Medical School, Boston; Fellowship – The Wilmer Ophthalmological Institute/The Johns Hopkins Hospital, Baltimore

Specialty Interests: Medical and surgical management of adult and pediatric glaucoma, adult cataract and intraocular lens implant surgery

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Specialty Training: Residency – American University of Beirut Medical Center, Beirut, Lebanon; Residency – Georgetown University Hospital, Washington, D.C.; Fellowship – Children’s Hospital National Medical Center, Washington, D.C.; Fellowship – Johns Hopkins Hospital, Baltimore

Specialty Interests: Ocular diseases of children, genetic eye diseases, strabismus, retinoblastoma, congenital cataracts, childhood glaucoma

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Specialty Interests: Refractive surgery, corneal transplantation
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