13th Annual Symposium
Visual Sciences Research Center

Friday, June 17, 2011
8 a.m. – 5 p.m.
Saturday, June 18, 2011
8:30 a.m. – 12

Wolstein Auditorium & Lobby

Full Day of Presentations by VSRC Faculty
With Keynote Speakers:

Dr. Rohit Varma
Professor of Ophthalmology & Preventive Medicine
University of Southern California

Dr. Natalie Afshari
Associate Professor of Ophthalmology
Duke University
Welcome
From the Chairman

Welcome to the 13th Annual Visual Sciences Research Center Symposium. As you listen to today’s lectures and view our poster presentations, you will see the breadth and depth of our research talent; the diversity in its discovery and the unity in the spirit of translational medicine.

We are fortunate enough to be the home of some of the nation’s most respected and recognized clinicians, researchers and academicians. It is because of our collaborative approach to medicine that we continue to succeed in our innovation and approach to advancing human health.

On behalf of my colleagues, we thank you for your support, participation and unwavering dedication to vision science.

With best regards,

Jonathan H. Lass, M.D.
Charles I Thomas Professor and Chairman
Department of Ophthalmology & Visual Sciences
Case Western Reserve University
Director, Case Visual Sciences Research Center
Director, University Hospitals Eye Institute

Contact Information
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Keynote Speaker
Rohit Varma, M.D.

Dr. Varma is Professor of Ophthalmology and Preventive Medicine, and Director of the Glaucoma Service, Ocular Epidemiology Center and the Clinical Trials Unit at the USC Keck School of Medicine. Dr. Varma earned his medical degree at the University of Delhi, India. He also obtained a Masters in Public Health from Johns Hopkins University. He completed his residency at the Wilmer Ophthalmological Institute at Johns Hopkins Hospital in Baltimore, Maryland and two glaucoma fellowships, one at the Wills Eye Hospital in Philadelphia, and the other at the Doheny Eye Institute, University of Southern California. His primary research focuses on epidemiologic studies of eye disease in children and aging populations. Dr. Varma is principal investigator of the Los Angeles Latino Eye Study (LALES), the Multi-Ethnic Pediatric Eye Diseases (MEPEDS) Study, and the Chinese American Eye Study (CHES). He is an expert on changes in the optic nerve in glaucoma, and is also studying new imaging techniques in the early diagnosis of glaucomatous optic nerve damage. More recently, Dr. Varma has been involved in the development of novel implantable IOP sensors and drainage devices. He has over 102 publications in various peer reviewed ophthalmic journals, and co-authored 2 ophthalmic books.

Academic Honors

- National Science Talent Award, 1980 - 1985
- Indian Council for Medical Research, 1982
- Goldberg Award, 1986 - 1989
- Fight for Sight Post-Doctoral Award, 1987 - 1988
- Fight for Sight Post-Doctoral Award, 1988 - 1989
- Dana Foundation Award, 1988 - 1989
- Topcon Corporation Award, 1988 -1989
- Delta Omega Society Award, 1989
- Topcon Corporation Award, 1991-1993
- National Eye Institute Travel Award, 1993
- National Eye Institute National Research Service Award, 1994 - 1997
- Fight for Sight Grant-in Aid Award, 1994 - 1995
- Knights Templar Grant Award, 1995 - 1996
- Beckman Young Investigators Award, 1996 - 1998
- Research to Prevent Blindness Career Development Award, 1997 - 2001
- American Academy of Ophthalmology Honor Award, 1998
- Research to Prevent Blindness Sybil B. Harrington Scholar Award, 2003
- American Academy of Ophthalmology Achievement Award, 2006

Education

MD– Maulana Azad Medical College – University of Delhi, Delhi, India 1986
Fellow - Glaucoma Service, Willis Eye Hospital, Philadelphia, PA 1986-89
Post Graduate Education M.P.H. – Johns Hopkins University, Baltimore, MD – Epidemiology 1989
Residency - Wilmer Ophthalmological Institute, Johns Hopkins Hospital, Baltimore, MD 1990-93
Fellow - Glaucoma Service, Doheny Eye Institute, Univ. of Southern California, Los Angeles, CA 1993-94

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Doheny Eye Institute
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Keynote Speaker
Natalie Afshari, M.D.

Dr. Afshari is a full-time faculty member at Duke University Eye Center, where she serves as both a clinician and a research scientist. Dr. Afshari’s achievement in cornea and refractive surgery and overall patient care has been recognized by her peers who have named her as one of “The Best Doctors in America” in Best Doctors, Inc.’s 2003-2004, 2005-2006 and 2007-2008, 2009-2010 editions. In addition, Dr Afshari was awarded the academic achievement award by the American Academy of Ophthalmology. The U.S. Food and Drug Administration (FDA) has sought Dr. Afshari’s expertise in evaluating ophthalmologic treatments, and the National Institutes of Health (NIH) has recognized Dr. Afshari and her collaborators to scientific research by awarding them a sizable grant to study Fuchs endothelial corneal dystrophy, a genetic disorder leading to corneal transplantation. In her clinical practice, Dr. Afshari specializes in cataract surgery, corneal transplantation, descemets stripping endothelial keratoplasty (DSEK), intacs for keratoconus, laser refractive surgery, including LASIK, LASEK/Advanced Surface Ablation, PRK, PTK, and treating surgical and medical diseases of cornea. She is a highly skilled cataract surgeon and holds national positions on the professional committees of cataract specialists, including serving as the American Society of Cataract and Refractive Surgery’s representative on the Council of the American Academy of Ophthalmology. Dr. Afshari is also a recognized expert in corneal transplantation and refractive surgery and has successfully treated many complex cases referred to her by other leading surgeons.

Academic Honors

- The Best Doctors in America 2003-04, 2005-06
- Best Doctors in North Carolina 2004, 2005
- Research to Prevent Blindness Research Award
- HEED Foundation Ophthalmic Award
- Teacher of the Year Award Duke University Eye Center
- Editorial Board Member, EyeNet
- American Society of Cataract and Refractive Surgery Committee
- American Society of Cataract and Refractive Surgery Committee
- Cataract Subspecialty Interest Team of American Academy of Ophthalmology
- Cataract and Anterior Segment Panel of American Academy of Ophthalmology
- Association for Research in Vision and Ophthalmology (ARVO) Cornea Program Committee
- Consultant to FDA Ophthalmic Drugs Panel

Education
MA, Endocrinology – University of California, Berkeley, 1991
MD – Stanford University School of Medicine, 1995
Residency – Internal Medicine, Harvard University, 1996
Residency – Ophthalmology, Harvard University, 1999
Residency – Cornea and Refractive Surgery, Harvard University, 2001

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The Visual Sciences Research Center

Who We Are:

The Visual Sciences Research Center (VSRC) was founded at Case Western Reserve University in 1996. The VSRC now comprises a multidisciplinary and comprehensive research program in vision and ophthalmology, with over 30 members in CWRU departments including Ophthalmology, Anatomy, Biomedical Engineering, Genetics, Medicine, Molecular Biology & Microbiology, Epidemiology & Biostatistics, Neurology, Neurosciences, Otolaryngology, Pathology, Pediatrics, Pharmacology, Physiology, and Biophysics. VSRC scientists study basic and clinical aspects of the eye and involve three interdisciplinary research theme groups, which include Aging and Diabetes, Retinal Degeneration, and Ocular Immunology.

Mission:

To promote the study of basic and clinical problems of the eye and visual system that may lead to improvements in the prevention and treatment of major blinding disorders worldwide. Through a multidisciplinary and comprehensive research program in vision and ophthalmology involving both basic and clinical departments at Case Western Reserve University, the VSRC seeks to advance the visual sciences at the University and beyond, and promote its efforts to the scientific community and greater community at large.

How We Make It Happen:

The VSRC is supported by a National Eye Institute (NEI) funded P30 Core Grant, directed by Dr. Eric Pearlman, and an NEI T32 Training Grant, directed by Dr. Susann Brady-Kalnay. Links to the P30 Core Grant and NEI T32 Training Grant can be found on the Department of Ophthalmology’s website at www.case.edu/med/ophthalmology

Please remember to acknowledge the P30 Core Grant on all publications and presentations: EY11373
The CORE Modules

Tissue Culture & Hybridoma
Core Managers:
Dawn Smith 368-0790 & Denice Major 368-6385
Provides facilities and assistance for the maintenance and propagation of ocular and other cell types.

Molecular Biology
Core Manager:
Anna Yakubenko 368-4990
Provides genotyping services, including primer design, and works with the Specialized Animal Resources Core to ensure you are working with the purest strains of mice available. Also assists with cloning and construct construction.

Microscopy & Digital Imaging
Core Manager:
Scott Howell 368-2300
Generates the highest quality images around, provides training for your microscopy needs, and designs automated microscopy routines and image analysis macros to enhance and speed up your research.

Histology
Core Manager:
Catherine Doller 368-5239
Produces high-quality paraffin or cryostat sections and slides, as well as histological stains.

Specialized Animal Resources
Core Managers:
Katie Franck & Heather Butler 368-5427
Provides genotyping services, including primer design, and works with the Specialized Animal Resources Core to ensure you are working with the purest strains of mice available. Also assists with cloning and construct construction.

Proteomics
Core Manager:
Benlian Wang 368-2599
Prepares your samples for Mass spec and provides extremely thorough analysis for your many proteomic needs.

Please remember to acknowledge the P30 Core Grant on all publications and presentations: EY11373
20th anniversary of the modern CWRU and University Hospitals Department of Ophthalmology: 
A Century of Accomplishments

The Department of Ophthalmology and Visual Sciences at Case Western Reserve University and University Hospitals Case Medical Center is pleased to celebrate its 20th anniversary of the establishment of the modern department in 1991. The Department was originally founded by Benjamin Millikin, MD, in 1893 at Lakeside Hospital and Western Reserve Medical School located in downtown Cleveland. Trained at the Wills Eye Hospital in Philadelphia and Germany, Dr. Millikin brought the latest advances in ophthalmology as a separate medical science to Northeast Ohio and also had a strong interest in medical student education in this discipline. He went on to serve as Dean of the School of Medicine from 1900-1912. He was succeeded as Ophthalmology chair in 1916 by William Bruner, MD, who also trained at Wills. Dr. Bruner served in this capacity until 1936. During this period he was one of the leaders in the founding of some of the enduring institutions for health care and education in Cleveland, including the Allen Medical Library, the Cleveland Sight Center, and most importantly the relocation of the Medical School and Lakeside Hospital in 1932 to create the modern academic medical center that exists today on Adelbert Road in University Circle.

In 1937, all the surgical specialties, including Ophthalmology, became divisions in the Department of Surgery under Dr. Carl Lenhart. Ophthalmology remained in a divisional structure for 54 years directed initially by Dr. Abram Bruner, nephew of Dr. William Bruner, until 1951. Notable during this period was the establishment of the residency program in 1937 with Dr. Charles I Thomas as the first resident. Dr. Thomas later became chairman of the Division in 1961 but prior to this Dr. Lornard Johnson, one of the founders of the Association for Research in Vision and Ophthalmology (ARVO), the largest organization fostering eye research in the world, led the Division. Dr. Johnson recruited the first full-time faculty member in the Division, Dr. Albert Potts, who went on to receive the Friedenwald Award, the top prize for a vision researcher given by ARVO for his work on methanol toxicity, and Chair of Ophthalmology at the University of Louisville and Arizona.

Then in 1961, Dr. Thomas, the first full-time chair, oversaw the expansion of the residency program from one to 5 residents per year with the inclusion of the VA Medical Center and MetroHealth Medical Center as affiliated hospitals of the program. He also founded the Cleveland Eye Bank, and the Johnson Laboratory for Vision Research including a retinal program under Dr. Ed Kean and ophthalmic ultrasound
Dr. Edward Purnell, a Case Western Reserve University School of Medicine and residency alum, assumed the chairmanship in 1973, continuing his seminal work on ophthalmic ultrasound, strengthening the residency program, leading expansion of clinical programs, and advancing a translational and clinical research under Dr. Jonathan Lass.

Nearing his retirement after 18 years as Division Chief, working with Dr. Lass, Dr. Purnell, with the support of Dr. Jerry Shuck, chair of Surgery, Dean Neil Cherniack, President Agnar Pytte, and Mrs. Farah Walters, president of University Hospitals of Cleveland, led the establishment of the modern Department of Ophthalmology by the University and University Hospitals in 1991. Two years later, Dr. Lass assumed the Chairmanship and became the second Charles I Thomas Professor. Under his leadership clinical programs have expanded with services in both general and all specialty areas, tripling of clinical faculty and facilities, including new outpatient facilities at Landerbrook, Westlake, and Hudson, and new surgical locations in Mayfield Heights and Westlake. Teaching programs have undergone tremendous changes with the residency program expanding to six residents per year with acquisition of the Mt. Sinai program (1995) and the St. Luke’s program (2000), and addition of St. Vincent Charity Hospital as an affiliate hospital under Dr. Dan Weidenthal’s and subsequently Dr. Marc Abrams’ leadership, and expansion at the VA in 2011 with new state of the art facilities and creation of two teams of residents under Dr. Ed Burney’s leadership. More than 275 residents have trained in the program since Dr. Thomas in 1937 with graduates now in practice throughout the country and their placement in top, highly competitive fellowships. The first international experience to the Prasad Eye Institute in Hyderabad, India was initiated in 2009 with the support of a local foundation, Ophthalmic Education Worldwide.

The basic research program expanded from one basic scientist to six with primary appointments and five with secondary appointments led by Dr. Eric Pearlman, Research Director. This expansion was facilitated by tripling of laboratory space and recruitment funds provided by the School of Medicine under Dean Ralph Horwitz and Dean Pamela Davis, and from University Hospitals under Dr. Fred Rothstein’s direction, and from generous donors to the Department. A driving force for the creation of an independent department was the department chair and faculty to be eligible for grants from Research to Prevent Blindness (RPB), the largest private foundation to fund vision research in the United States. The Department entered the unrestricted RPB grant program in 1997 and since has received prestigious individual awards (Career Development, Wasserman, Senior Investigator, Medical Student, and the Jules and Doris Stein Professorship), reflecting the highest quality of vision science of our researchers. The Department’s basic science efforts were enhanced with the approval by Dean Nathan Berger in 1996 of the creation of the Case Visual Sciences Research Center under Dr. Lass’ direction which has grown to 18 basic and clinical departments involved in vision research.
research and more than $9 million/year in federal funding with NIH P30 Core Grant and T32 Training grant funding continuously from 1997.

The clinical research program in the Department expanded with the founding of the Vision Research Coordinating Center in 2004 and Clinical Trials Unit under Dr. Suber Huang’s leadership with double digit growth in corporate and federal trials, investigator initiated studies, and multicenter studies including the National Eye Institute sponsored Fuchs’ Genetics Multicenter Study. The last decade showed major growth in the two image analysis reading centers in the Department, the Cornea Image Analysis Reading Center and the Retinal Image Analysis Reading Center (REDIARC), conducting both corporate and federal studies requiring analysis of corneal and retinal images in a controlled manner, overseen by their technical director, Beth Ann Benetz. Finally, with a solid philanthropic foundation from previous chair efforts, the Department has added three additional endowed chairs (Asseff, Searle-Huang, and Page-Reinhart), two major research funds (Russell, Mitchell), and three named lectureships (Purnell, Bruner, and Levine). The excellence and depth of all these clinical, teaching, and research programs led to the designation by University Hospitals of our Department as an Eye Institute in 2008.

The legacy of excellence since Dr. Millikin’s aspirations to develop ophthalmology as a separate discipline at Western Reserve School of Medicine and Lakeside Hospital in 1893 to the founding of the modern Department of Ophthalmology in 1991 should serve as inspiration for the next generation of clinicians, educators and researchers with our Department and our Eye Institute.

Jonathan Lass, M.D.
Charles I Thomas Professor and Chairman
CWRU Department of Ophthalmology and Visual Sciences
Director, University Hospitals Eye Institute
Director, Case Visual Sciences Research Center
June 2011
20th anniversary of the modern CWRU and University Hospitals
Department of Ophthalmology:

Western Reserve School of Medicine - 1890s

Lakeside Hospital in downtown Cleveland – 1890s

Department Leadership Since 1890s

Benjamin Milich MD 1893-1913
William Bruner, MD 1916-1936
Abram Bruner MD 1937-1951
Lorand Johnson MD 1951-1963
Charles Thomas MD 1961-1973
Edward Purnell MD 1973-1993
Jonathan Lass MD 1993-
Dr. Thomas with Dr. Purnell, who founded ocular ultrasonography laboratory with Dr. Thomas’ support.

Jerry Shuck, M.D.
Chair, Dept of Surgery
Supported the establishment of modern Department of Ophthalmology

Edward Kean, Ph.D. who had continuous funding from National Eye Institute for over 20 years under Drs. Thomas and Purnell, examining the chemistry of the visual pigment, rhodopsin.
Western Reserve School of Medicine and University Hospitals of Cleveland: A Blueprint for the new academic medical center - 1922

UH Case Medical Center Campus – 2011
13th Annual Symposium
Visual Sciences Research Center
Schedule

**Friday, June 17, 2011**

7:30  Continental Breakfast, Poster Setup

8:00  **Jonathan H. Lass, M.D.**  
Charles I Thomas Professor and Chairman, Ophthalmology & Visual Sciences  
*Opening Remarks*

8:10  **Eric Pearlman, Ph.D.**  
Professor and Director of Research, Ophthalmology & Visual Sciences  
*Update on Visual Sciences Research Center*

**Retinal Biology Moderator: Shawn Wilker, M.D.**

8:20  **Saida Omarova, M.D.**  
Postdoctoral Researcher, Ophthalmology and Visual Sciences  
Irina Pikuleva, Ph.D. Lab  
*The Unexpected Link Between Cholesterol and Retinal Neovascularization*

8:30  **Alejandro Colozo, Ph.D.**  
Research Associate, Ophthalmology and Visual Sciences  
Paul Park, Ph.D. Lab  
*Comparison of the Molecular Interaction that Stabilize Bovine and Mouse Rhodopsin*

8:45  **Akiko Maeda, M.D., Ph.D.**  
Assistant Professor, Ophthalmology & Visual Sciences  
*Primary amines protect the retina from degeneration in mouse models: implications for Stargardt’s disease and age-related macular degeneration*

9:00  **Elisa Bala, M.D.**  
Resident, Ophthalmology & Visual Sciences  
*Multifocal Electroretinography and Genetic Correlations in Early Age-Related Macular Degeneration*

9:15  **Johannes von Lintig, Ph.D.**  
Assistant Professor, Pharmacology  
*Molecular Components Affecting Ocular Vitamin A Homeostasis*
9:30  **Sanae Sakami, Ph.D.**  
Postdoctoral Researcher, Pharmacology  
Krzysztof Palczewski, Ph.D. Lab  
*Probing Mechanisms of Photoreceptor Degeneration in a New Mouse Model of the Common Form of Autosomal Dominant Retinitis Pigmentosa due to P23H Opsin Mutations*

9:45  **Feng Lin, Ph.D.**  
Assistant Professor, Pathology  
*Myeloid suppressor cells induced by retinal pigmentation epithelial cells inhibit autoreactive T cell responses that lead to experimental autoimmune uveitis*

### Aging and Diabetes Moderator: Vincent Monnier, M.D.

10:00  **Ram Nagaraj, Ph.D.**  
Carl F. Asseff, M.D. Professor of Ophthalmology & Visual Sciences, Pharmacology  
*Inflammatory cytokines induce the expression of indoleamine 2,3-dioxygenase in human retinal capillary endothelial cells: Implications for diabetic retinopathy*

10:15  **Xingjun Fan, Ph.D.**  
Instructor, Pathology  
*Mouse Models of Accelerated Aging by Carbonyl and Oxidant Stress*

10:30  **Virginia Utz, M.D.**  
Resident, Ophthalmology & Visual Sciences  
*Spectral Domain-OCT Imaging of Melanocytic Lesions*

10:45  **Carlos Subauste, M.D.**  
Associate Professor, Medicine  
*CD40 is Central for Development of Diabetic Retinopathy*

### 20th Anniversary Presentation, Department of Ophthalmology & Visual Sciences

11:10  **Jonathan H. Lass, M.D.**  
Charles I Thomas Professor and Chairman, Ophthalmology & Visual Sciences

11:20  **Jerry Shuck, M.D., D.SC**  
Associate Dean and Director of Graduate Medical Education

11:30  **Pamela B. Davis, M.D., Ph.D.**  
Dean, School of Medicine

**Lunch & Poster Session**
### Epidemiology & Biostatistics Moderator: Sudha Iyengar, Ph.D.

1:00  
**Keynote Speaker**  
**Rohit Varma, M.D.**  
Professor of Ophthalmology & Preventive Medicine  
Director of Glaucoma Service, Ocular Epidemiology Center and the Clinical Trials Unit  
Doheny Eye Institute, University of Southern California  
*LALES (Los Angeles Latino Eye Study) and MEPEDS (Multi-Ethnic Pediatric Eye Disease Study) – Lessons to Improve Prevention of Visual Impairment and Blindness in the United States*

2:00  
**Keynote Speaker**  
**Natalie Afshari, M.D.**  
Associate Professor of Ophthalmology  
Director of Cornea and Refractive Surgery  
Duke University  
*Genetics of Corneal Dystrophies and Gene Therapy in Cornea*

3:00  
**Sudha Iyengar, Ph.D.**  
Professor, Epidemiology & Biostatistics  
*Identification of Novel Loci for Diabetic Retinopathy in European Americans: The Family Investigation of Nephropathy and Diabetes (FIND)*

### Cornea Moderator: Jonathan Lass, M.D.

3:15  
**Gary Wnek, Ph.D.**  
The Joseph F. Toot, Jr., Professor of Engineering  
Professor of Macromolecular Science and Engineering  
Faculty Director, The Institute for Management and Engineering (TiME)  
*A Versatile Type I Collagen Processing Platform*

3:30  
**Brian McDermott, Ph.D.**  
Assistant Professor, Otolaryngology  
*Fascin 2b Is a Component of Stereocilia that Lengthens Actin-Based Protrusions*

3:45  
**M. Edward Medof, M.D., Ph.D.**  
Professor, Pathology, Medicine, Ophthalmology, and Cancer Center  
*Mechanisms of Ocular Tolerance*

### Visual Sciences Training Program Moderator: Susann Brady-Kalnay, Ph.D.

4:00  
**Patricia R. Taylor, Ph.D.**  
Postdoctoral Researcher, Ophthalmology & Visual Sciences  
Eric Pearlman, Ph.D. Lab  
*An essential role for T cell and neutrophil derived IL-17 in fungal keratitis*
4:15  Debarshi Mustafi  
MSTP Student, Pharmacology  
Krzysztof Palczewski, Ph.D. Lab  
*Defective photoreceptor phagocytosis in a mouse model of Enhanced S-cone Syndrome causes progressive retinal degeneration*

4:30  Casey Charvet  
Ph.D. Student, Ophthalmology & Visual Sciences  
Irina Pikuleva, Ph.D. Lab  
*Post-translational Modification of CYP27A1 by Oxidative Derivatives of Arachidonic Acid, Isolevuglandins, Occurs in Human Retina.*

4:45  Steven de Jesus Carrion  
Ph.D. Student, Ophthalmology & Visual Sciences Dr. Eric Pearlman Lab  
*Surface hydrophobins in fungal spores promote pathogenicity during Fungal Keratitis*

**Poster Session & Reception**

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**Saturday, June 18**

8:30  Continental Breakfast

9:10  Shawn Wilker, M.D.  
Opening Remarks

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**Retina Moderator: Shawn Wilker, M.D.**

9:15  Mehul Nagarsheth, M.D.  
Resident, Ophthalmology & Visual Sciences  
*Incidence and severity of Retinopathy of Prematurity in premature Infants with birth weight > 1250 grams and/or gestational age > 28 weeks*

9:30  Johnny Tang, M.D.  
Assistant Professor, Ophthalmology & Visual Sciences  
*Increased Corneal Thickness in Patients with Ocular Coloboma*
Cornea Moderator: Kristina Thomas, M.D.

9:45  Keynote Speaker
   Natalie Afshari, M.D.
   Associate Professor of Ophthalmology
   Director of Cornea and Refractive Surgery
   Duke University
   Advances in Corneal Surgery

10:45  Break

11:00  Donghai Ho, M.D.
   Resident, Ophthalmology & Visual Sciences
   Susceptibility of Fungal Biofilms on Worn Soft Contact Lenses to Lens Care Solutions

11:10  Loretta Szczotka-Flynn, O.D., Ph.D.
   Professor, Ophthalmology & Visual Sciences and Epidemiology & Biostatistics
   Application of Activated Protein C in Reducing Soft Contact Lens Associated Fungal Biofilms

11:20  Scott Yeates, M.D.
   Resident, Ophthalmology & Visual Sciences
   Corneal Topographic Changes During Continuous Silicone Hydrogel Contact Lens Wear

11:30  Jonathan H. Lass, M.D.
   Charles I Thomas Professor and Chairman, Ophthalmology & Visual Sciences
   Update on Corneal Transplant Studies: Cornea Donor Study, DSEK, & Future Plans

11:45  Holly Simpson, M.D.
   Resident, Ophthalmology & Visual Sciences
   Comparison of Nidek Automated, Automated Adjusted, and Manual Center Methods of Corneal Endothelial Cell Analysis

12 – 3 p.m.  Resident Graduation Festivities: Dr. Jonathan Lass, Dr. Johnny Tang, Marianne Reeves

Thank You for Attending
The 13th Annual
Visual Sciences Research Center Symposium
## Presenter Contact Information in Order of Appearance

<table>
<thead>
<tr>
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<th>Title/Role</th>
<th>Contact Information</th>
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**Title:** The Unexpected Link Between Cholesterol and Retinal Neovascularization

**Purpose:** To evaluate the effects of CYP27A1 deficiency, the major retinal cholesterol hydroxylase, on visual function in mice.

**Methods:** We utilize optical coherence tomography (OCT), fluorescein angiography, and histology for evaluation of retinal morphology, and electroretinography (ERG) to assess visual function.

**Results:** At the age of 1.5 mo, CYP27A1 knockout (KO) mice begin to develop bilateral retinal pathology in the inferior retina as revealed by OCT. By the age of 12-mo, some of the KOs exhibit pathology both in the inferior retina and near the optic nerve. The pathology starts from the choroid and penetrates through Bruch’s membrane and RPE into the outer retina. Doppler flow OCT indicates abnormal blood flow in CYP27A1 KOs. Fluorescein angiography in CYP27A1 KOs reveals blood leakage in the same area showing pathology on OCT. We observe transient statistically significant increases in the amplitude of the photopic b-wave in 3 mo CYP27A1 female KOs as compared to WT littermates by ERG. Male CYP27A1 KOs also have statistically significant increases in the photopic b-wave amplitude, but starting later, at 6 mo.

**Conclusion:** CYP27A1 KO mice develop a bilateral retinal pathology starting at 1.5 mo old, primarily in the inferior retina. At the age of 12 mo, more CYP27A1 KO males (80%) are affected than females (67%).

Doppler-OCT and fluorescein angiography reveal altered blood flow and leakage, respectively, in the retina of CYP27A1 KO mice.

CYP27A1 KO mouse retina is more hypoxic as assessed by immunohistochemistry studies primarily in the retinal GCL, OPL, and IS of the photoreceptors.

CYP27A1 KO mice develop neovascularization, mainly in the inferior retina as shown by H&E, wheat-germ agglutinin, VEGFR-2, VEGF staining.

CYP27A1 KO male mice at 8 mo old have complement deposition (C3), suggestive of inflammatory processes in the GCL, OPL, and choriocapillaris.

ERG indicates that responses are gender- and genotype-specific. Increased amplitudes may be a result of tissue hypoxia due to impaired blood flow.
Abstract Information

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**Title:**
Single molecule force spectroscopy as a tool for understanding the molecular interactions that stabilize rhodopsin

**Purpose:**
Rhodopsin is the light receptor that initiates phototransduction. Over 100 different point-mutations have been identified in the receptor that lead to retinal dystrophies. It therefore is important to understand the molecular interactions that underlie the various physiologically relevant states of rhodopsin, particularly those that cause disease.

**Methods:**
Single-molecule force spectroscopy (SMFS) is a technique that can mechanically unfold single rhodopsin molecules in near native-conditions. It can quantify and localize the forces that stabilize the structure of the receptor. Using SMFS, wild-type mouse rhodopsin was unfolded from native disc membranes of rod photoreceptor cells and compared to that of bovine rhodopsin.

**Results:**
Nine major unfolding intermediates were detected that define the most stable segments in both receptors. The location and occurrence of those segments essentially are identical between bovine and mouse. Moreover, by mechanically unfolding rhodopsin at different pulling velocities via dynamic SMFS, the kinetic stabilities as well as the mechanical properties of each of those nine segments can be determined. No appreciable differences can be detected between bovine and mouse.

**Discussion and Conclusion:**
The results suggest that the twenty-three residues that differ naturally between bovine and mouse bear little consequence on the molecular interactions that stabilize the structures of both receptors. The present work, therefore, establishes a framework for the unfolding spectra of wild-type mouse rhodopsin for use in future studies to interpret the unfolding properties of mutant rhodopsins from transgenic mice, which are now used ubiquitously as model systems for human retinal disease.
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**Title:** Primary amines protect the retina from degeneration in mouse models: implications for Stargardt’s disease and age-related macular degeneration

**Abstract:** Visual perception is triggered in the retina by the absorption of light by the visual pigment chromophore, 11-cis-retinal, which then is continuously regenerated through a series of reactions termed the visual cycle. However, toxic side reactions occur, especially those involving reactive aldehyde groups of the photoisomerization product, all-trans-retinal, and they can cause severe retinal pathology. Here we transiently lowered free all-trans-retinal with primary amine-containing approved drugs that do not inhibit chromophore regeneration in mouse models of retinal degeneration. Schiff base adducts between all-trans-retinal, and these amines were identified by mass spectrometry, and their levels in the eye were proportional to the capability of the drug to preserve the retina in short-term and long-term experiments. This study demonstrates the molecular basis of the pathology and identifies a family of approved compounds and their protective effects on the retinal degeneration that display feature of Stargardt’s and age-related macular degeneration.
13th Annual Symposium – Visual Sciences Research Center

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**Title:** Multifocal Electroretinography and Genetic Correlations in Early Age-Related Macular Degeneration

**Purpose:** To determine if genetic risk factors play a role in retinal dysfunction noted in AMD patients as assessed using standard multifocal electroretinography (mfERG).

**Methods:** A total of 62 male subjects, 28 patients and 34 unrelated age-matched controls, were included in our study. Patients were diagnosed through ophthalmologic examination, and subcategorized by criteria defined by the Age-Related Eye Disease Study (AREDS). All patients included in the study exhibited clinical features of mild to moderate ARMD, namely categories 2 and 3. Controls lacked macular drusen and exhibited no clinical evidence of any retinal disorder. DNA was genotyped by direct genomic sequencing or RFLP analysis for AMD risk alleles that together account for ~75% of the combined AMD genetic risk (CFH (rs1061170), HTRA1 (rs11200638), ARMS2 (rs10490924), C3 (rs2230199)). Standard mfERGs were recorded on all subjects; responses were analyzed in concentric ring averages and N1 and P1 amplitudes and P1 latency of the average waveforms were noted.

**Results:** Patients with a greater number of AMD risk alleles were more likely to have delayed mfERG responses from the foveal and parafoveal areas. When individual genes were evaluated, mfERG delays were more pronounced for patients carrying 1 or 2 risk alleles for HTRA1 and ARMS2, while there was no clear relationship for C3 or CFH. There was also no clear relationship between mfERG amplitude and genetic risk alleles.

**Conclusions:** Delayed mfERGs have been previously reported in AMD patients. In this study, we note that risk alleles for some AMD-related genes (HTRA1, ARMS2) are more strongly associated with mfERG delays than others (C3, CFH). This suggests that the different genetic risk factors may manifest with different pathophysiological signatures.
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**Title:** Molecular components affecting ocular vitamin A homeostasis

**Purpose:**
The essential role of vitamin A for the vertebrate eye has long been known because deficiency in or excess of this vitamin can impair eye development and function. Vitamin A is the precursor for at least two critical metabolites, 11-cis-retinal, the chromophore of visual pigments, and retinoic acid, a ligand for transcription factors that are required for eye development. Ocular vitamin A uptake from the serum retinol binding protein (RBP) is a homeostatic process that evidently depends on specific transporters and metabolic enzymes. Mutations in these genes are associated with disease, including Retinitis pigmentosa and the fatal Mathew-Wood Syndrome (MWS). The objective of our research was to elucidate the pathway for ocular vitamin A supply, including its regulation, by genetic dissection in animal models.

**Methods:**
We used zebrafish and mouse models to respectively analyze the role of STRA6 (stimulated by retinoic acid gene 6) and LRAT (lecithin:retinol acyltransferase) for ocular retinoid uptake and blood vitamin A homeostasis. We analyzed the consequences of deficiency of these components by using molecular biology and biochemical methods.

**Results:**
In a zebrafish model for MWS, we demonstrated that vitamin A uptake from RBP essentially depends on STRA6. In STRA6-deficiency of these eyes, vitamin A homeostasis was disrupted and caused retinoic acid excess. In mouse models, we showed that uptake of vitamin A from blood RBP is dependent on esterification by LRAT. Moreover, we provide evidence that both STRA6 and LRAT activity is subject to regulation of retinoic acid signaling.

**Discussion:**
Our studies in animal models provide evidence that STRA6 and LRAT play essential roles in the eyes to acquire vitamin A from the blood. Our findings demonstrated that vitamin A homeostasis must be tightly controlled to avoid imbalances in the production of the visual chromophore and retinoic acid that both play essential roles in eye development and functioning.

**Conclusion:**
Our studies provided new insights into the control of ocular vitamin homeostasis. These findings will improve understanding of human disease states caused by disturbances in this process. The established animal models can be used to develop concepts for prevention and therapy of such disease.
**Title:** Probing Mechanisms of Photoreceptor Degeneration in a New Mouse Model of the Common Form of Autosomal Dominant Retinitis Pigmentosa due to P23H Opsin Mutations

**Purpose:** A P23H mutation in the opsin gene is one of the most prevalent causes of the human blinding disease, autosomal dominant retinitis pigmentosa. Although P23H cultured cell and transgenic animal models have been developed, there remains controversy over whether they fully mimic the human phenotype; and the exact mechanism by which this mutation leads to photoreceptor cell degeneration remains unknown. To explore the mechanism of P23H-induced disease, we generated novel P23H rhodopsin knock-in mice to model the disease in human patients and examined the mechanistic details of this mutation.

**Methods:** Retinal phenotype of humans with autosomal dominant retinitis pigmentosa caused by the P23H was assessed by psychophysical testing, electroretinography and retinal cross-sectional imaging. Retinal phenotype of P23H mice was assessed by immunohistochemistry, light microscopy, transmission electron microscopy and electroretinography. Glycosylation of P23H protein and protein level was analyzed by immunoblotting in P23H mouse. Effect of chromophore on cytotoxicity of P23H protein was analyzed by genetic ablation of chromophore production in P23H mouse.

**Results:** Rod photoreceptors in our P23H knock-in mice displayed regional degeneration and decreased lengths of their rod outer segment, but most survived for several months, even though their outer segment structures were compromised. This phenotype mimics changes in the retina of patients with autosomal dominant retinitis pigmentosa caused by the P23H opsin mutation. P23H protein was inadequately glycosylated with levels 1–10% that of wild type opsin. Moreover, the P23H protein failed to accumulate in rod photoreceptor cell endoplasmic reticulum but instead disrupted rod photoreceptor disks. Genetic ablation of chromophore production accelerated retinal degeneration in these mice.

**Discussion:** In P23H opsin-transfected cultured cells, P23H opsin accumulated and formed aggregates in the endoplasmic reticulum. Whether tissue culture experiments accurately mimic human retinal conditions and how the relative dosage of the WT and mutant opsin affects rod outer segment integrity and retinal pathology in transgenic animal models have been topics of debate. By using our animal model, we found that the mouse P23H protein was inadequately glycosylated with levels between 1 and 10% that of wild type opsin. Moreover, the P23H protein did not accumulate in the endoplasmic reticulum, as described in some transgenic rodent opsin-mutant models, but instead disrupted the rod photoreceptor disks. These data indicate that photoreceptors may have degenerated due to disorganization of outer segment.

**Conclusion:** Generated P23H opsin knock-in mice appear to be the most suitable model to date for further study of human autosomal dominant retinitis pigmentosa retinal pathology and disease mechanisms and for proof-of-concept studies of potential therapies. Our results indicate that most synthesized P23H protein is degraded, and its retinal cytotoxicity is enhanced by lack of the chromophore.
Title: Myeloid suppressor cells induced by retinal pigmentation epithelial cells inhibit autoreactive T cell responses that lead to experimental autoimmune uveitis

Research: Retinal pigmentation epithelial cells (RPE) suppress local immune reactions by secreting cytokines and by expressing PD-L1 on their cell surface to directly inhibit T and B cell responses, which contribute to the ocular immunoprivileged status, and help to control inflammation in many retinal diseases. Previous studies demonstrated that myeloid-derived suppressor cells (MDSC) are present in the retina in experimental autoimmune uveitis (EAU), a model of autoimmune posterior uveitis. However, whether local retinal cells are able to induce the differentiation of MDSC to control inflammatory reactions, and if so, what the underlying mechanisms are, remain completely unknown. In this report, we demonstrated that RPE induce the differentiation of MDSC from bone marrow progenitor cells, in which both RPE cell surface molecules and secreted factors are integrally involved. The RPE-induced MDSC significantly suppress autoreactive T cell responses that lead to retinal injury in EAU. These data revealed a novel mechanism by which RPE suppress immune reactions, which may contribute to the inflammation control in the retina. These results also provide insights into the development of new methods to generate large numbers of syngeneic MDSC for the treatment of autoimmune posterior uveitis, and more broadly, for the treatments of other similar autoimmune diseases as well as the prevention of transplanted grafts rejection.
Inflammatory cytokines induce the expression of indoleamine 2,3-dioxygenase in human retinal capillary endothelial cells: Implications for diabetic retinopathy
Maneesh Mailankot and Ram H Nagaraj, Department of Ophthalmology and Visual Sciences, Case Western Reserve University, Cleveland, OH, USA

Purpose: Indoleamine 2,3-dioxygenase (IDO) is the key regulatory enzyme in the kynurenine pathway. Several studies including our own have shown that IDO is induced by interferon-γ and that kynurenines produced through IDO-mediated oxidation of tryptophan are cytotoxic. It is now well established that inflammatory cytokines play a role in the pathogenesis of diabetic retinopathy, although the mechanisms are still not clear. In this study we investigated the effect of inflammatory cytokines on IDO and determined the biochemical mechanisms by which kynurenines induce apoptosis in human retinal capillary endothelial cells (HREC).

Methods: Human eyes were obtained from the Cleveland Eye Bank. HREC in culture were treated with TNF-α (0-20 ng/ml) or IL-1β (0-20 ng/ml) for 2 days. IDO activity was determined by HPLC, and IDO protein expression by immunocytochemistry and western blotting. Intracellular kynurenines levels and protein modification by kynurenines were assessed by HPLC and immunocytochemistry, respectively. The involvement of p38MAPK signaling pathway was studied by western blotting. ROS generation in cells was detected by a fluorescence assay and cell cycle analysis by flow cytometry. The role of IDO in cytokine-induced cytotoxicity was evaluated in the presence specific chemical inhibitors of the kynurenine pathway.

Results: The IDO activity and protein expression were higher in the diabetic than in the non-diabetic human retina. The immunocytochemical analyses showed higher IDO expression in capillary endothelial cells of the diabetic retina. TNF-α and IL-1β dose dependently induced IDO protein expression and enzyme activity in HREC in culture. Chemical inhibition of p38MAPK activation blocked cytokine-induced IDO expression. The IDO induction was accompanied by an increase in kynurenine formation, protein modification by kynurenine, ROS generation and cell cycle perturbation. All these cytotoxic effects were blocked by 1-methyl D,L-tryptophan, a competitive inhibitor of IDO.

Conclusion: Our results suggest that IDO expression and activity are higher in the diabetic human retina and IDO-mediated kynurenine formation could be a potential mechanism for retinal capillary endothelial cell death in the diabetic retina.

Support: R01EY-016219, R01EY-09912, P30EY-11373, RPB, OLERF
**Purpose:** Aging and most age-related diseases are associated with progressive accumulation of protein damage in lens, collagen-rich tissues, neurodegenerative diseases and many other conditions that have in common a progressive weakening of antioxidant defenses. Both diabetes and end stage renal disease worsen these changes. While the nature of the protein changes due to glycation and oxidation are getting better understood, it has been difficult to directly implicate them in the disease process itself.

**Methods:** In order to close this gap, our laboratory is developing novel animal mouse models of accelerated tissue aging, by selectively overexpressing or knocking out genes of importance for the control of carbonyl and oxidant stress. The first model was the hSVCT2 mouse in which we showed that expression of the human vitamin C transporter 2 in the lens could mimic and accelerate the protein modifications observed during lenticular aging (Fan et al. PNAS 2006). Two new models of accelerated protein aging by oxidation have now have been generated by conditionally targeting glutathione synthesis in lens (LEGSKO mouse) (in order to mimic the low GHS levels in the core of the human lens) and nerve (NEGSKO mouse).

**Results:** Gamma glutamyl cysteine ligase mRNA, activity and glutathione (GSH) levels are severely depressed in the lens of the homozygous mouse. Methionine oxidation and protein disulfide formation in lens crystallins are already highly increased at 6 mos of age. Mice develop nuclear opacities as in the aging human lens. In the NEGSKO mouse, molecular, cellular and behavioral phenotypes resembling amyotrophic lateral sclerosis develop at one month of age, which include low pan-neuronal levels of GSH, increased apoptosis, diminished neuronal cell count, increased tau phosphorylation, and cleavage of the ALS protein marker TDP-43.

**Conclusions:** The availability of these novel disease models is expected to be highly fruitful for the development of drugs against age-related cataract and ALS.
CD40 is central for development of diabetic retinopathy.

Purpose: CD40 is a member of the TNF receptor superfamily expressed on various cell types. CD40 has been linked to certain inflammatory and neuro-degenerative disorders making CD40 a therapeutic target for these disorders. It is not known if CD40 drives micro-vascular complications of diabetes such as diabetic retinopathy.

Methods: B6 and CD40ko mice were made diabetic by streptozotocin administration. Leukostasis, retinal mRNA levels of ICAM-1, MCP-1 and CD40 as well as histopathology were examined. Protein levels of these molecules were assessed by flow cytometry, ELISA or immunoblot.

Results: Compared to diabetic B6 mice, diabetic CD40ko mice exhibited diminished upregulation of ICAM-1 and MCP-1. These mice also had a marked reduction in retinal leukostasis and did not develop degenerate capillaries. CD40 is expressed on primary human and mouse Muller cells, retinal endothelial cells and microglia. CD40 expression is increased in retinas of diabetic B6 mice. In vitro studies revealed that CD154 (CD40 ligand) upregulated ICAM-1 expression and MCP-1 secretion by human retinal endothelial and Muller cells, as well as NOS2 expression by human Muller cells. CD40 stimulation of Muller cells caused apoptosis of bystander retinal endothelial cells.

Discussion: These studies indicate that CD40 is an important regulator of pro-inflammatory responses in the retina during diabetes and promotes capillary degeneration. Enhanced CD40 signaling may be triggered during diabetes by the upregulation of CD40 in the retina, the increased serum levels of CD154 (CD40 ligand) reported in patients with diabetic retinopathy as well as by the presence of microthrombi in the retina (platelets express CD154).

Conclusion: These studies provide the first evidence that CD40 is key for the development of diabetic retinopathy.
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**Title**: Identification of Novel Loci for Diabetic Retinopathy in European Americans: The Family Investigation of Nephropathy and Diabetes (FIND)

**Purpose**: Diabetic Retinopathy (DR) is a common microvascular complication of type 1 and 2 diabetes mellitus (DM). It affects over 60% of diabetic individuals, and is a leading cause of visual impairment. DR ranges in severity from mild/moderate non-proliferative disease to proliferative disease, and is often accompanied by macular edema. In the hopes of gaining insights into DR susceptibility, we conducted a genome-wide association study (GWAS) in a European American sample with and without DR.

**Methods**: In FIND, participants had ophthalmological exams and stereoscopic seven-field retinal photos graded, and assessed according to the methods of the Early Treatment Diabetic Retinopathy Study Group (ETDRS). DNA from participants was genotyped using the Affymetrix 6.0 chip with ~ 950,000 markers, imputed to >2.4 million markers. Logistic regression was used to contrast cases with DR (N=105) versus controls (N = 627) who were disease free, adjusting for the effects of sex, study center, DM duration, and two principal components for population structure. Glycosylated hemoglobin was examined as a covariate, but not included because it was not significant.

**Results**: The best p-value was on chromosome 20 at rs2425644 (Odds Ratio = 3.0; p = 1.2 x 10-7) within the serine incorporator 3 locus (SERINC3). SERINC3 is a transmembrane protein whose function has not been well characterized. In addition to SERINC3, several other loci with similar evidence on chromosomes 2, 3, 4, 5 and 14 were also observed. Genotyped and imputed markers in previously reported candidate genes such as EPO, VEGFA and its receptor KDR showed no association at p<0.05.

**Conclusions**: Our analysis has identified several novel loci for DR that may provide insights into DR etiology. We are pursuing replication of these results in additional samples in order to provide better support for these findings.
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**Title:** A Versatile Type I Collagen Processing Platform

**Purpose:** The major aim of this work has been to develop Type I collagen formulations that allow for easy fabrication into a variety of useful 3-D materials including nanofiber scaffolds, gels, lyophilized foams, contact lenses, and printable patterns. Applications may include wound repair (skin, cornea), 3-D cell culturing, and multi-functional scaffolding for use in regenerative medicine. Also, therapeutic molecules can be incorporated into the solutions prior to processing which can be later released.

**Methods:** Type I collagen (Semed-S, Kensey-Nash Corp.) can be processed from benign solvent systems such as ethanol/PBS in the presence of the zero-length crosslinking system EDC-NHS with kinetics tuned to delay crosslinking for several hours. Electrospinning is carried out by methods previously reported from this laboratory (Dong et al., *Macromol. Rapid. Commun.*, **30**, 539 (2009)). Gels can be fabricated using the same solutions by allowing the crosslinking to occur over a few hours. Foams can be prepared by lyophilizing the crosslinked gels.

**Results:** Electrospun, crosslinked nanofibers of Type I collagen (mean diameter ca. 200 nm) are readily fabricated from 1:1 PBS:ethanol solutions containing EDC and NHS (EDC:NHS = 1:2); see Figure 1.

![Figure 1. A) SEM of electrospun collagen; B) macroscopic mat](image)

**Discussion:** Two key points: (1) Acid-soluble, Type I collagen can be solubilized in ethanol/PBS to concentrations as high as 20mg/ml which is sufficient for facile electrostatic spinning into sub-micron-diameter fibers; (2) the kinetics of a common zero-length crosslinking system, EDC-NHS, can be sufficiently delayed using NHS/EDC > 1 such that spinning can be achieved in the presence of the crosslinker. Both the benign solvent system and delayed-reaction crosslinker can be used for the fabrication of other collagen constructs, including gels, tubes, and lyophilized foams.

**Conclusion:** Type I collagen can be simply processed into many useful forms using a novel, benign solvent system, ethanol/PBS, coupled with a kinetically-controlled crosslinking system. Applications in ophthalmology may include corneal wound healing, especially in conjunction with release of therapeutically-active molecules.
Title: Fascin 2b Is a Component of Stereocilia that Lengthens Actin-Based Protrusions

Purpose: Present examples of zebrafish technology to study the ear and the eye.

Stereocilia are actin-filled protrusions that permit mechanotransduction in the internal ear. To identify proteins that organize the cytoskeleton of stereocilia, we scrutinized the hair-cell transcriptome of zebrafish. One promising candidate encodes fascin 2b, a filamentous actin-bundling protein found in retinal photoreceptors. Immunolabeling of zebrafish hair cells and the use of transgenic zebrafish that expressed fascin 2b fused to green fluorescent protein demonstrated that fascin 2b localized to stereocilia specifically. When filamentous actin and recombinant fusion protein containing fascin 2b were combined in vitro to determine their dissociation constant, a $K_d \approx 0.37$ M was observed. Electron microscopy showed that fascin 2b-actin filament complexes formed parallel actin bundles in vitro. We demonstrated that expression of fascin 2b or espin, another actin-bundling protein, in COS-7 cells induced the formation of long filopodia. Coexpression showed synergism between these proteins through the formation of extra-long protrusions. Using phosphomutant fascin 2b proteins, which mimicked either a phosphorylated or a nonphosphorylated state, in COS-7 cells and in transgenic hair cells, we showed that both formation of long filopodia and localization of fascin 2b to stereocilia were dependent on serine 38. Overexpression of wild-type fascin 2b in hair cells was correlated with increased stereociliary length relative to controls. These findings indicate that fascin 2b plays a key role in shaping stereocilia.
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**Title:** An essential role for T cell and neutrophil derived IL-17 in fungal keratitis

**Research:** Fungal keratitis caused by filamentous *Aspergillus* and *Fusarium* species is one of the major causes of corneal ulcers in the USA and in the developing world. Our studies indicate that CD4+ cells are present in the corneas of infected individuals; therefore, we examined the role of adaptive immunity in fungal keratitis. C57BL/6 mice were immunized subcutaneously with heat killed *A. fumigatus* or *F. oxysporum* spores (conidia), and 100,000 live conidia were injected into the corneal stroma. After 24, 48, or 72 h, corneas were dissected, homogenized, and cell lysates were examined by cytokine arrays and ELISAs. Alternatively, corneas were digested with collagenase, and cells were examined by flow cytometry. We found that immunization led to rapid clearance of fungi and resolution of disease compared with unimmunized mice. The response was associated with infiltrating neutrophils at 24 h, and recruitment of CD4+/CD3+ cells 48 and 72h post-infection. Intracellular staining showed IL-17 producing neutrophils at 24 hrs, CD4+/IL-17 producing cells 48 hrs, and CD4+/IFN-g producing cells 72 hrs after infection. The protective immune response was ablated after CD4 cell depletion, and conferred after adoptive transfer of CD4 cells from immunized mice. Furthermore, local neutralization of IL-17 reversed protective response. Together, these results demonstrate that Th17 cells have an essential role in the host response to fungal keratitis.
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**Title:** Defective photoreceptor phagocytosis in a mouse model of Enhanced S-cone Syndrome causes progressive retinal degeneration

**Purpose:** Enhanced S-cone syndrome (ESCS), featuring an excess numbers of S-cones, manifests as a progressive retinal degeneration leading to blindness. Although ESCS was identified in humans decades ago and since then the causative genes have been elucidated, our understanding of the accompanying retinal degeneration is still poorly understood. Through genetic and structural characterization, we sought the first insight into the disease pathophysiology leading to the progressive retinal degeneration in ESCS.

**Methods:** Massively parallel RNA-sequencing was carried out to decipher the eye and retinal transcriptomes of wild type mice and mice with knockout of the Nrl transcription factor, a phenotypic model of ESCS. The genetic findings were complemented by novel high-resolution scanning electron microscopy (SEM) imaging was used to probe the photoreceptor-retinal pigmented epithelium interface. Transmission electron microscopy (TEM) revealed high-resolution views of the abnormal retinal structures brought on by ESCS that were then biochemically verified with confocal microscopy.

**Results:** RNA-Seq experiments revealed 6203 differentially expressed transcripts between wild type and Nrl-/ mouse retinas. This provided new insight into the transcriptional mis-regulation in the ESCS murine model and revealed a change in gene expression in putative proteins involved in photoreceptor phagocytosis. Structurally characterization of wild type and ESCS murine model retinas showed that the phagocytotic defect was due to the inherent defect in the photoreceptors stemming from their aberrant development. A detailed study of the photoreceptor-RPE interface with high resolution imaging methods such as SEM and TEM revealed abnormal interactions of ESCS photoreceptors with the RPE and a build-up of vacuole-like material in the heads of the photoreceptor.

**Discussion:** Researchers have wrestled with the human ESCS disease and its animal model to try to elucidate the molecular mechanism for photoreceptor degeneration in this developmental retinopathy and we accomplished this with both a comprehensive sequencing method and a variety of cellular imaging modalities, some of which had yet to be used in visual science research. Both the defect in phagocytosis and the degenerative component of ESCS seem attributable to aberrant photoreceptors in the retina rather than a combination of photoreceptor and RPE cell dysfunction.

**Conclusion:** The inherent defect in phagocytosis in the Nrl-/ retina observed in this work is likely caused by changes in the normal transcriptional landscape that causes an overpopulation of ESCS photoreceptors in the retina. Our extensive analysis of ESCS suggests that successful treatment strategies in this disease could include means to improve the phagocytotic defect in order to arrest the progressive degeneration component of the disease.
Abstract Information

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**Title:** Post-translational Modification of CYP27A1 by Oxidative Derivatives of Arachidonic Acid, Isolevuglandins, Occurs in Human Retina.

**Purpose:** Cytochrome P450 27A1 (CYP27A1) is an important contributor to the enzymatic elimination of cholesterol from the retina. To determine whether activity of this enzyme is decreased with age and in age-related macular degeneration, we applied multiple reaction monitoring (MRM) mass spectrometry assays to characterize the oxidized lipid modification of CYP27A1 in human retina.


**Discussion:** Isolevuglandins promiscuously modify protein amino groups and likely affect the function of CYP27A1 and other enzymes in vivo. Further, studies in vivo have shown isolevuglandin modified proteins are resistant to degradation and can elicit immunogenic and cytotoxic responses, thus leading to impaired cellular health. We propose that increased oxidation of PUFA's represents a common link between aging/oxidative stress/inflammation and AMD as well as cardiovascular disease (CVD).

**Conclusion:** By utilizing MRM, we demonstrate for the first time post-translational modification of a protein by iso[4]LGE$_2$ in the human retina and that oxidative stress can potentially affect cholesterol homeostasis in the retina. The MRM methodology developed creates a paradigm for similar studies on other proteins.
Fungal infections of the cornea (i.e keratitis) are caused mainly by filamentous fungi, with *Fusarium* and *Aspergillus* species being the most common agents. Fungal keratitis occurs predominantly in developing nations causing up to 60% of corneal infections. The air we constantly breathe is filled with spores from these ubiquitous organisms and still most of the times we show no signs of infection. It has been shown that *A.fumigatus* spores produce a unique protein known as rodA that coat the conidial surface, making it inert to the immune system. This finding led us to propose that surface rodA protein (rodAp) provides time for the spore to germinate and cause disease in the cornea during fungal keratitis infection. To explore this, we injected C57Bl/6 mice corneas with $5 \times 10^4$ rodA-/- conidia from *A.fumigatus* and observed higher fungal killing by 24h post-infection, which was accompanied by higher leukocyte infiltration at 24 and 48h. Chemical removal of surface rodA (using hydrofluoric acid) in several strains isolated from patients with keratitis showed exposure of β-glucan and stimulated pro-inflammatory cytokine production in macrophages. Human Neutrophils exposed to live rodA-/- exhibited higher ability to kill the fungus compared to the Wt strain, indicating that even after spore germination rodA-/- hyphae are more readily recognized and killed. These findings suggest that surface hydrophobins do more than just coat the conidial surface and can enhance fungal pathogenicity in the cornea during fungal keratitis, although the mechanism remains unknown.
Abstract Information

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**Project Mentor(s)**

**Authors:** Mehul H Nagarsheth; Alison S Smith; Dashminder Singh; Faruk H Orge; Jeffrey N Bloom

**Title:** Incidence and severity of Retinopathy of Prematurity in premature Infants with birth weight > 1250 grams and/or gestational age > 28 weeks

**Purpose:** Retinopathy of prematurity (ROP) is a vision-threatening disorder of preterm infants. Current guidelines recommend that selected infants with birth weight 1500-2000 g or gestational age more than 32 weeks with an unstable clinical course should have retinal screening examinations to detect ROP. In our study, we reviewed ROP examinations in our Level III NICU to determine upper limits of birth weight and gestational age for screening purposes.

**Methods:** A retrospective chart review was performed for all premature infants admitted from January 1, 1997-September 30, 2008. 950 charts from this time period were reviewed. Infants with birth weights greater than 1250 grams and/or gestational age greater than 28 weeks were noted and followed for regression or treatment of ROP. Particular attention was paid to infants with birth weight greater than 1500 grams and/or gestational age greater than 30 weeks.

**Results:** We identified 71 subjects with birth weights of 1250 grams or greater, 33 subjects with birth weights equal to or greater than 1500 grams, and 152 subjects born at 28 weeks gestational age or greater. None of the 33 subjects with birth weights greater than or equal to 1500 grams required laser ROP treatment.

**Discussion:** Our data suggest that, unlike current guidelines above, a birth weight upper limit < 1500 grams is unlikely to miss cases of ROP requiring laser therapy.

**Conclusion:** With a screening birth weight upper limit of 1500 grams, ophthalmologists would perform less ROP screening examinations. This in turn would reduce health-care cost and physical stress to premature infants.
**Abstract Information**

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<tr>
<th>Presenter Name</th>
<th>Johnny Tang, M.D.</th>
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**Authors:** Johnny Tang and Brian P. Brooks

**Title:** Increased Corneal Thickness In Patients With Ocular Coloboma

**Purpose:** Uveal coloboma is a developmental abnormality of the eye caused by failure of the optic fissure to close during the fifth week of gestation. To characterize the anatomy of colobomatous eyes, we have analyzed the central corneal thickness (CCT) in 4 consecutive patients diagnosed with ocular coloboma.

**Methods:** This was a consecutive case series of 4 patient with uveal coloboma. Each patient underwent full ophthalmological examination including measurements of their CCT.

**Results:** We describe significantly increased CCT in 3 of 4 consecutive patients with ocular coloboma.

**Discussion:** In all four cases, the corneas of our patients appeared to be of normal thickness clinically; however, corneal pachymetry demonstrated significantly increased CCT in 3 of the 4 patients. Increased CCT may artifactually elevate IOP on Goldmann applanation tonometry. These observations should be born in mind before instituting glaucoma treatments in coloboma patients with apparently elevated IOP.

**Conclusion:** Our study highlights the need to consider the measurement of CCT in patients presenting with ocular colobomas.
Abstract Information

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**Title**: Susceptibility of Fungal Biofilms on Worn Soft Contact Lenses to Lens Care Solutions

**Purpose**: To assess the efficacy of marketed contact lens care products against *Fusarium* biofilms formed on worn contact lenses using an established in-vitro soft contact lens-*Fusarium* biofilm model.

**Methods**: Two *Fusarium* strains (*Fusarium solani* 6914 and *Fusarium oxysporum* 8996) from two patients with fungal keratitis were incubated with three different types (lotrafilcon A, etafilcon A, and balafilcon A) of worn contact lenses from three different subjects under conditions that facilitate biofilm formation. Biofilm was quantified using a tetrazolium XTT [2,3-bis (2-methoxy-4-nitro-5-sulphophenyl) -2H -tetrazolium -5 -carboxanilide] assay. Susceptibilities of the biofilm growth phases of the fungi to five common multipurpose (MPS) contact lens care solutions (three polymeric biguanide-preserved and two polyquaternium-preserved) and two hydrogen peroxide care systems were assessed. Effective antifungal activity was defined if the care product achieved >=50% reduction in the metabolic activity of treated biofilms, compared to phosphate-buffered saline-soaked, worn controls.

**Results**: Both *Fusarium solani* and *Fusarium oxysporum* strains formed biofilms on worn lotrafilcon A, etafilcon A, and balafilcon A contact lenses. The biofilms of *F. solani* on all three lens types were consistently susceptible to both hydrogen peroxide care systems (growth reduction of 84-97%, p<=0.01) and two of the five MPS (growth reduction of 62-85% for a biguanide-preserved MPS, p<=0.01; growth reduction of 92-96% for a polyquaternium-preserved MPS, p<0.01). The biofilms of *F. oxysporum* on all three lens types were consistently susceptible to both hydrogen peroxide care systems (growth reduction of 79-99%, p<=0.005) and one of the five MPS (growth reduction of 93-96% for a polyquaternium-preserved MPS, p<=0.004).

**Conclusion**: *F. solani* and *F. oxysporum* form biofilms on all three types of worn contact lenses, which are resistant to the antifungal activity of several soft contact lens care products. Only the hydrogen peroxide care systems and one polyquaternium-preserved MPS consistently demonstrated effective antifungal activity against both *Fusarium* strains on all three lens types.
Abstract Information

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**Title:** Application of Activated Protein C in Reducing Soft Contact Lens Associated Fungal Biofilms

**Purpose:** Fibrin is a key component of biofilms acting as a conditioning layer or matrix during biofilm development. Activated Protein C (APC) is a potent physiologic anticoagulant with profibrinolytic properties. This study assessed the activity of APC against soft contact lens associated fungal biofilms using an in-vitro established soft contact lens fungal biofilm model.

**Methods:** *Fusarium solani* 6914 and *Fusarium oxysporum* 8996 were incubated with three different types of worn contact lenses (lotrafilcon A, balafilcon A, and etafilcon A) from three different subjects under conditions that facilitate biofilm formation. Both strains were obtained from patients with fungal keratitis. Biofilm was quantified using a tetrazolium XTT \(2,3\)-bis (2-methoxy-4-nitro-5-sulfophenyl) -2H -tetrazolium -5 -carboxanilide\] assay. Susceptibilities of the fungal biofilm growth phases to 25 ug/ml of APC solution were assessed under two separate conditions: when the APC solution was added during the adhesion phase of biofilm development or after 48 hours of mature biofilm formation.

**Results:** APC was not effective against *F. solani* biofilm formation when added during the adhesion phase. However, when added after mature biofilm had been formed, APC significantly reduced *F. solani* biofilm activity by 69%, 72%, and 81% for etafilcon A, lotrafilcon A, and balafilcon A lenses, respectively, compared to phosphate buffered saline-soaked worn-lens controls \((p<0.05)\). *F. oxysporum* biofilm activity was reduced by 26%, 1%, and 1% on etafilcon A, lotrafilcon A, and balafilcon A lenses, respectively, compared to phosphate buffered saline-soaked worn-lens controls.

**Discussion:** APC has potent antifungal activity against a *F. solani* soft contact associated biofilm using an XTT-based metabolic activity assay. There is variability in its effect as it was ineffective against *F. oxysporum* or when added prior to mature biofilm development. Nevertheless, further study is warranted as APC is an agent that may have novel anti-biofilm activity and it has potential to be used in combination with contact lens disinfecting products to reduce contact lens associated biofilms.

**Support:** Prevent Blindness America
Title: Corneal Topographic Changes During Continuous Silicone Hydrogel Contact Lens Wear

Purpose: To assess whether continuous wear (CW) with silicone hydrogel (SH) lotrafilcon A contact lenses induces changes in Orbscan II-derived corneal topographic indices.

Methods: Subjects were fitted with lotrafilcon A lenses for monthly CW and observed for 1 year. Orbscan II measurements of right eyes were taken at baseline, and after 1 week (n=151), 1 month (n=151), and 1 year (n=85) of wear. Slit images were reprocessed using a beta version (v 3.12) edge tracker software and the following indices were evaluated: anterior steep and flat simulated keratometry (simK), posterior corneal best fit sphere, posterior corneal elevation, and pachymetry at the thinnest 0.5mm corneal spot. Paired T-tests were utilized stratified by type of contact lens worn at study entry.

Results: No changes were noted in posterior corneal indices or pachymetry over time. For the entire cohort, significant changes were noted for anterior steep and flat simK values after 1 week of CW. Average steep simK flattened by 0.20 D (p=0.0016, range -4.14 to +2.79 D), 0.27 D (p<0.0001, range -4.11 to +2.82 D), and 0.29 D (p=0.003, range -5.12 to +1.78 D), after 1 week, 1 month and 1 year, respectively. Stratified by pre-study contact lens use, neophytes (n=47) showed no simK changes over time; SH lens wearers (n=24) showed the greatest amount of corneal flattening of 0.44 D, 0.38 D, and 0.59 D after 1 week, 1 month and 1 year, respectively (p<0.007); and low Dk lens users (n=71) had significant flattening at all time points of 0.21 D, 0.35 D and 0.30 D after 1 week, 1 month, and 1 year respectively, (p<0.039) and the greatest variability of all groups (range -5.12 D to +2.87 D).

Conclusions: CW of lotrafilcon A lenses did not alter the thinnest pachymetry, posterior corneal elevation or posterior best fit sphere in this sample. Subjects switching from low Dk lenses had the greatest variation in simK values, but subjects switching from other SH lens types had the greatest amount of average corneal flattening. These findings are valuable for clinicians when monitoring patients for refractive shifts during SH contact lens CW.
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Title: Update on Corneal Transplant Studies: CDS, DSEK vs PKP, and future studies

Purpose: Overview of corneal transplant studies over past decade to examine factors impacting graft survival following penetrating and endothelial keratoplasty and describe plans for future studies

Methods: Prospective randomized clinical trials examining effect of donor age with secondary analyses on donor, operative and perioperative factors impacting graft survival and endothelial cell loss following penetrating (Cornea Donor Study) and endothelial keratoplasty for endothelial dysfunction conditions. 5 year followup for penetrating keratoplasty patients and 3 years for EK cases. Similar methods to be applied for examination of preservation time on graft success and endothelial cell loss following EK.

Results: Donor age found to not significantly impact graft survival following PKP for endothelial dysfunction conditions while cell loss significantly greater with donors over 65 at 5 years. Donor factors do not affect graft survival but donor gender, graft size and age do. Pseudophakic bullous keratopathy and presence of glaucoma major risk factors for graft failure. EK graft survival comparable to PKP graft survival, while cell loss is greater in first year but comparable by third year to PKP cell loss.

Discussion and Conclusions: Results suggest the value of long term followup and a multi-center prospective masked approach to the conduct of these studies to determine the impact of these factors on graft survival. In regards to PKP, with the differences noted in graft survival vs. cell loss will be important to get 10 year data. In regards to EK, remarkable differences in type and rate of cell loss with comparable graft survival to PKP, also suggest the importance of long term followup in a multicenter approach from academic and private practice clinical sites, similar to the CDS, to determine the value of EK in the larger community. A study is proposed to examine preservation time in this setting that can assist in answering these questions.

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**Title**: Comparison of Nidek Automated, Automated Adjusted, and Manual Center Methods of Corneal Endothelial Cell Analysis

**Purpose**: To compare corneal endothelial cell image analysis using the Konan KSS-300 center method and the Nidek automated endothelial analysis system with and without manual correction.

**Methods**: Twenty five randomly selected images of good to excellent quality of the central corneal endothelium were obtained from a sample of images by confocal microscopy performed with a Nidek Confoscan 4 (40x objective) collected as part of a study of the corneal effects of dosing taprenepag isopropyl (PF-04217329) qAM for 14 days. The study included 32 patients (24 non-Japanese and 8 Japanese) with a prior history of glaucoma or ocular hypertension, normal slit lamp exam, and no history of ocular trauma, intraocular surgery or intraocular inflammation. Images were captured at baseline, days 7 and 13 on treatment, and day 35. Identical images were analyzed using the Konan KSS-300 center method and the Nidek automated endothelial analysis system provided by the manufacturer (Nidek Technologies, Inc., Greensboro, NC) with (Nidek automated-adjusted) and without manual correction (Nidek automated). All three methods of image analysis were used to determine endothelial cell density (ECD) as well as coefficient of variation (CV) and % of hexagonal cells (%HEX) and were statistically evaluated for differences by the Student's paired t-tests.

**Results**: The mean ECD (± SO) determined using the Nidek automated (2468 ± 384 cells/mm²) image analysis software was higher but not statistically Significant when compared to the ECD determined with the Konan KSS 300 center method (2355 ± 552 cells/mm²). The Nidek automated-adjusted (2346 ± 561 cells/mm²) was equivalent to the KSS-300 center method. The mean CV was significantly higher for Nidek automated analysis method (p= 0.001) while the %HEX was significantly lower (p= 0.01) when compared to the Konan method. The Nidek automated-adjusted CV (p= 0.27) and %HEX (p= 0.64) was equivalent to the KSS-300 center method.

**Conclusions**: The Nidek automated endothelial image analysis software with and without manual editing (automated-adjusted) produces results comparable to the manual Konan KSS-300 center method for determination of ECD but requires manual adjustment for other morphometric parameters.
Thank you for attending our 13th Annual VSRC Symposium.

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