Program and Abstracts

International Conference on the Lens

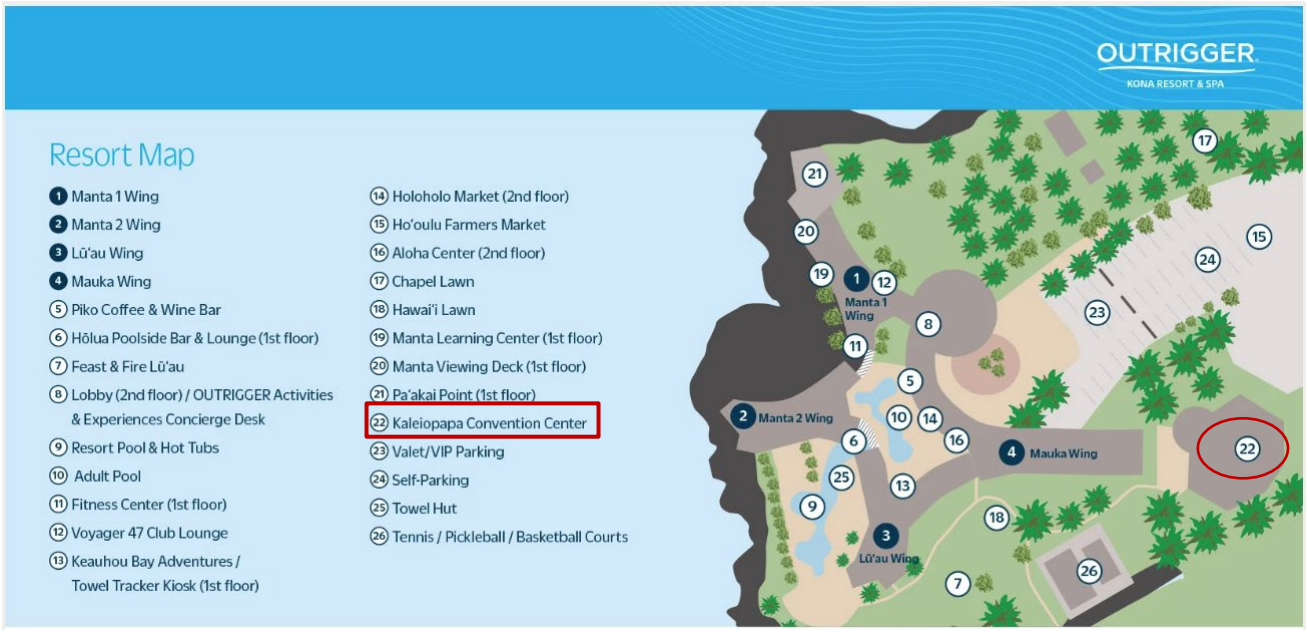
Kailua Kona, Hawaii
December 7-12, 2025

OUTRIGGER KONA RESORT & SPA

[Resort website]

[Resort Map]

+



ICE MACHINES & FILTERED WATER DISPENSERS (END OF THE HALLWAYS)

- 1 2 Manta 1 and Manta 2 — FLOORS ..... 2 / 3 / 4
- 3 Lū'au — FLOORS ..... 1 / 2 / 3 / 4
- 4 Mauka ..... 2 / 3 / 4 / 5 / 6 / 7

ICE MACHINES (BEHIND THE ELEVATOR & END OF HALLWAY)

- 4 Mauka ..... 3 / 4 / 5 / 6 / 7

FOOD & BEVERAGE SERVICES

⑤ **Piko Coffee & Wine Bar**
Open Daily
Bar // Grab & Go ..... 6:00 am - 10:00 pm
Breakfast ..... 6:00 am - 10:30 am
Dinner ..... 5:30 pm - 9:00 pm
Live Music ..... 6:00 pm - 9:00 pm

⑥ **Hōlua Poolside Bar & Lounge**
Open Daily
Drinks ..... 11:00 am - 7:00 pm
Lunch ..... 11:00 am - 5:00 pm
Pupu ..... 5:00 pm - 7:00 pm

⑦ **Feast & Fire Lu'au Show**
Monday & Thursday
Dinner & Show 5:00pm

RESORT SERVICES

⑧ **OUTRIGGER Activities & Experiences Concierge (ext. 75157 & 75158)**
Available also by phone or email
DAILY ..... 8:00 am - 5:00 pm
Scan QR code to book activities and Feast & Fire Lū'au

⑨ **Resort Pool & Hot Tubs**
ALL AGES ..... 8:00 am - 10:00 pm
SUPER SLIDE ..... 9:00 am - 6:00 pm

⑩ **Adult Pool**
AGES 21+ ..... 8:00 am - 10:00 pm

⑪ **Fitness Center**
DAILY ..... Room key

⑫ **Voyager 47 Club Lounge**
OPEN DAILY ..... 7:00 am - 9:30 am and 4:30pm - 6:30 pm

⑬ **Keauhou Bay Adventures (ext. 5104)**
OPEN DAILY ..... 8:00 am - 7:00 pm
Towel Tracker Kiosk (Adult Pool)
OPEN NIGHTLY ..... 7:00 pm - 10:00 pm

⑭ **Holoholo Market**
OPEN DAILY ..... 6:00 am - 10:00 pm

⑮ **Ho'oulu Farmers Market**
WEDNESDAYS & FRIDAYS ..... 9:00 am - 2:00 pm

⑯ **Aloha Center**
CULTURAL ACTIVITIES
Scan QR code for reservations and schedules

⑲ **Manta Center**
FLOOR 1

㉕ **Towel Hut**
OPEN DAILY ..... 8:00 am - 7:00 pm

ICL 2025 – MEETING AT A GLANCE

Sunday, December 7th

3:00 pm – 6:00 pm	*Registration @ Kaleiopapa Convention Center*
6:00 pm	**Plenary Lecture - Noboru Mizushima**
7:00 pm – 9:00 pm	*Welcome Reception @ Kaleiopapa Convention Center Grounds*

Monday, December 8th

7:30 am	*Registration @ Kaleiopapa Convention Center*
8:15 am	Welcoming Remarks
8:30 am	**Session 1: Lens Cell and Matrix Biology**
10:00 am	Break – Tea/Coffee
10:30 am	**Session 2: Lens Differentiation**
12:00 pm	*Lunch*
1:15 pm	**Session 3: Structural Biology and Biophysics**
2:45 pm	*Break – Tea/Coffee*
3:00 pm	**Session 4: Cataract Genetics**

Tuesday, December 9th

8:30 am	**Session 5: Lens Signaling**
10:00 am	*Break – Tea/Coffee*
10:30 am	**Session 6: Crystallin Modifications & Cataractogenesis**
12:00 pm	*Lunch*
1:15 pm	**Session 7: Lens Clinical Studies**
2:45 pm	*Break – Tea/Coffee*
3:00 pm	**Early Career Cataract Research Award Presentation**
3:05 pm	**NFER Cataract Award Talk**
3:30 pm	**Poster Lightning Talks**
4:15 pm	**Poster Session**

Wednesday, December 10th

8:30 am	**Session 8: Redox Biology & Cataract**
10:00 am	*Break – Tea/Coffee*
10:30 am	**Session 9: Lens Optics & Biomechanics**
12:00 pm	***Afternoon Free / Golf Tournament***

Thursday, December 11th

8:30 am	**Session 10: Lens Membranes & Physiology**
10:00 am	*Break – Tea/Coffee*
10:30 am	**Session 11: Lens Development & Regeneration**
12:00 pm	*Lunch*
1:15 pm	**Session 12: PCO, Fibrosis, and EMT**
2:45 pm	*Break – Tea/Coffee*
3:00 pm	**Session 13: Crystalline Lens Optical Evaluation; Inspiration and Guidance for IOLs and Presbyopia Treatment**
4:30 pm	*Break*
5:30 pm	**The Kinoshita Lecture**
6:30 pm – 9:00 pm	***Conference Banquet at Bayview Grounds***

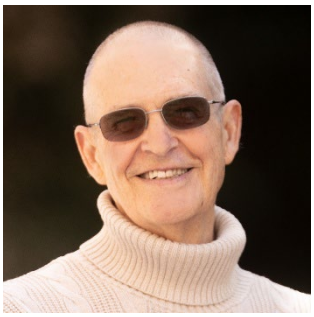
Friday, December 12th

8:30 am	**Session 14: Molecular Aging in Lens and Non-Lens Tissues**
10:00 am	*Break – Tea/Coffee*
10:30 am	**Session 15: No Lens is an Island**
12:00 pm	**Closing Remarks and Depart**

TABLE OF CONTENTS

NFER AWARDS ICL 2025

Kinoshita Lectureship



John I. Clark, PhD
University of Washington
Seattle, WA, USA

Cataract Research Award

Xiangjia Zhu, MD, PhD
Fudan University
Shanghai, China

Early Career Cataract Research Award

Eugene Serebryany, PhD
Stony Brook University
Stony Brook, NY, USA

TRAVEL AWARDS ICL 2025

Sponsored by NFER and the Japanese Cataract Group.

Henry Fukui Award

Salil Lachke, PhD
University of Delaware
Newark, DE

Frederick Bettelheim Award

Alberto Castro, PhD
Instituto de Optica, CSIC
Madrid, Spain

Alvira and Venkat Reddy Award

Takashi Ono, MD, PhD
University of Tokyo
Tokyo, Japan

Gerald Robison Award

Christina Karakosta, PhD
National and Kapodistrian University
Athens, Greece

J. Fielding Hejtmancik Award

Gavin Arno, PhD
Greenwood Genetic Center
Greenwood, SC, USA

Young Investigator Travel Awards

Promita Deb, MS
University of Delaware
Newark, DE

JudiRae DeDreu, MS
Thomas Jefferson University
Philadelphia, PA

Eduardo M Martinez-Enriquez, PhD
Instituto de Optica, CSIC
Madrid, Spain

Preston Hazen, BS
Boise State University
Boise ID

Xue-Bin Hu, PhD
Zhongshan Ophthalmic Center
Guangzhou City, China

Lanping Jiang, MSc
University of Auckland
Auckland, New Zealand

Constance Kraay, AM
Harvard University
Cambridge, MA

Qing Li, PhD
Shanghai Jiao Tong University
Shanghai, China

Anna Lubertozzi, BS
Ohio State University
Columbus, OH

Yoichiro Masuda, MD, PhD
Jikei University
Tokyo, Japan

Haruka Matsumoto, MD
Dokkyo Medical University
Mibu, Japan

Nikhil Nair, MSc
Auckland University
Auckland, New Zealand

Taishi Painter, BA
University of California Berkeley, Berkeley, CA

Yu Pu, BS
Stony Brook University
Stony Brook, NY

Pooja Rathaur, PhD
Washington University
St. Louis, MO

Takayoshi Sumioka, MD, PhD
Wakayama University
Wakayama, Japan

Yuyan Shan, MSc
Zhongshan Ophthalmic Center
Guangzhou, China

Sarah Zelle, BS
Vanderbilt University
Nashville, TN

INTERNATIONAL CONFERENCE ON THE LENS

A small interactive meeting focused on the biology of the normal lens and lens-related diseases.

Organizing Committee

Scientific

Steven Bassnett, Ph.D.
Washington University School of Medicine
St. Louis, MO, USA

Kevin Schey, Ph.D.
Vanderbilt University
Nashville, TN, USA

Administrative

Peter F. Kador, Ph.D.
Meredith, New Hampshire

Thomas White, Ph.D.
SUNY Stony Brook

J. Samuel Zigler, Jr., Ph.D.
Middletown, Maryland

Acknowledgements

We gratefully acknowledge support for the meeting received from:

NFER
THE National Foundation for Eye Research

PROGRAM

All Sessions held in Kaleiopapa Convention Center

Sunday, December 7th	
3:00 pm – 6:00 pm	*Registration @ Kaleiopapa Convention Center*
	Opening Session @ Kaleiopapa Convention Center
6:00 pm	**Plenary Lecture** Noboru Mizushima: Intracellular degradation by autophagy and PLAAT family lipases
7:00 pm – 9:00 pm	*Welcome Reception @ Kaleiopapa Convention Center Grounds*

Monday, December 8th	
7:30 am	*Registration @ Kaleiopapa Convention Center*
8:15 am	**Welcoming Remarks**
8:30 am	**Session 1: Lens Cell and Matrix Biology** *Cathy Cheng & TJ Plageman*
	1. TJ Plageman: Arvcf-dependent cell adhesion at lens sutures
	2. Justin Parreno: F-actin reorganization as a regulator of epithelial-mesenchymal transition in a serum/growth-factor free ex vivo model of lens fibrosis
	3. Juliet Moncaster: Microtubule-Associated Protein Tau (MAPT) in mouse non-transgenic and transgenic Alzheimer's Disease lenses
	4. Matthew A. Reilly: The Lens' Gradient Refractive Index Deteriorates Upon Removal of the Capsule and Epithelium
	5. Cathy Cheng: Eph-ephrin signaling affects lens growth and shape, nucleus size, and gradient refractive index in adult mice
10:00 am	*Break – Tea/Coffee*
10:30 am	**Session 2: Lens Differentiation** *Amer Riazuddin & Salil Lachke*
	1. Amer Riazuddin: The role of autophagy in lens fiber cell differentiation
	2. Michael L. Robinson: FOXE3 transcriptional targets suggest a role for FOXE3 in suppressing neural differentiation
	3. Jared Tangeman: Leveraging single-cell multiomics to resolve gene regulatory networks governing lens fiber cell differentiation
	4. Velia Fowler: Delayed Fiber Cell Maturation in CAP2cKO Mouse Lenses
	5. Salil Lachke: Ell2 is necessary for optimally accessible chromatin in early differentiation stages to achieve high levels of select transcripts in fiber cells
12:00 pm	*Lunch*

Monday, December 8th	
1:15 pm	**Session 3: Structural Biology and Biophysics** *Rachel Martin & Tamir Gonen*
	1. Rachel Martin: Biophysical characterization of site-specifically oxidized lens proteins
	2. Shane Gonen: CryoEM imaging methodology for difficult protein targets
	3. Rachel Klevit: Parsing the grammar of small heat shock proteins
	4. Tamir Gonen: MicroED solution to the lens MP20 enigma
	5. Haruka Matsumoto: Analysis of cataract pathophysiology analysis at the molecular level using mass spectrometry imaging
2:45 pm	*Break – Tea/Coffee*
3:00 pm	**Session 4: Cataract Genetics** *Elena Semina & Anne Slavotinek*
	1. Elena Semina: Uncovering new genetic factors in pediatric cataracts
	2. Gavin Arno: Nanopore sequencing transcript analysis and functional investigation of a novel CYP51A1 genotype confirms the gene's association with autosomal recessive congenital cataract
	3. Hélène Choquet: Novel cataract risk loci identified from a large-scale multiethnic genome-wide association meta-analysis
	4. Fielding Hejtmancik: Aspartyl (Asparaginyl) beta-Hydroxylase deficiency impairs lens placode invagination and lens development in a Traboulsi syndrome Mouse Model
	5. Anne Slavotinek: Single cell RNA-Seq in a zebrafish model of crim1 loss of function – uncovering gene regulatory networks and mechanisms for lens development

Tuesday, December 9th	
8:30 am	**Session 5: Lens Signaling** *Xin Zhang & David Li*
	1. David Li: Regulation of Lens Development by Pax6 Sumoylation
	2. Haotian Lin: Digital Twin of the Lens and AI-Assisted Diagnosis and Treatment of Cataracts
	3. Xiangjia Zhu: Pathogenetic Mechanisms of Highly Myopic Cataract
	4. Qian Wang: Dynamic regulation of FGF signaling in lens development
	5. Michael Karin: Oxidized mitochondrial DNA and systemic autoimmune diseases
10:00 am	*Break – Tea/Coffee*

Tuesday, December 9th	
10:30 am	**Session 6: Crystallin Modifications & Cataractogenesis** *Kirsten Lampi & Eugene Serebryany*
	1. Eugene Serebryany: Investigating the mechanism of suppression of γ-crystallin misfolding and aggregation by the abundant lens metabolite myo-inositol
	2. Adam Miller: α-crystallins play the long game
	3. Laxman Mainali: Aggregation of α-Crystallin to Lens Membrane Increases with Peroxidation of Lipid and Cholesterol
	4. Constance Kraay: Unfolding Intermediates of γD-Crystallin and γS-Crystallin Identified *in silico* Provide Insights into Mechanisms and Suppression of Aggregation
	5. Kirsten Lampi: Stepwise Mechanism of Crystallin Insolubilization due to the Synergistic Effects of Deamidation and Oxidation
12:00 pm	*Lunch*
1:15 pm	**Session 7: Lens Clinical Studies** *Hiroshi Sasaki & Eri Kubo*
	1. Hiroyuki Matsushima: Biocompatibility and Functionality of Intraocular Lens Materials
	2. Yoichiro Masuda: From Atopic Cataract to IOL Dislocation: A Pathological Journey Through the Lens Capsule
	3. Andrew Smith: LEFTY2 is an Aqueous Humor Biomarker for Exfoliation Glaucoma
	4. Takayoshi Sumioka: Dead Bag Syndrome after Cataract Surgery: Pathological Findings and a Representative Case
	5. Takashi Ono: Optimizing Systemic Care in Cataract Surgery for the Super-Aged Society
2:45 pm	*Break – Tea/Coffee*
3:00 pm	**Early Career Cataract Research Award Presentation**
3:05 pm	**NFER Cataract Award Talk (Dr. Xiangjia Zhu)**
3:30 pm	**Poster Lightning Talks (Nick Delamere)**
4:15 pm	**Poster Session**
	1. Venkata Pulla Rao Vendra: The cataract associated mutation G165R of human beta B3-crystallin destabilizes the molecule and promotes aggregation
	2. Keke Zhang: Comparative Phosphoproteomic Profiling of Lenses from Patients with Highly Myopic Cataract and Age-Related Cataract
	3. Hongzhe Li: Proteomic characterization of capsular fibrosis and the role of NPC1 in TGF-β2-induced epithelial-mesenchymal transition in lens epithelial cells
	4. Atsushi Tohma: Immunohistochemical Characteristics of Advanced Glycation End-products (AGEs) in the Lens Capsule in Intraocular Lens Dislocation
	5. Rylee King: The Role of Actin Binding protein, TAGLN, in Myofibroblast Differentiation of Lens Epithelial Cells

Tuesday, December 9th	
	6. Anna Lubertozzi: Afadin Orchestrates Tricellular Adherens Junctions and Lens Tissue Architecture
	7. Electra Coffman: Arvcf stabilizes adherens junctions and Ankyrin-B protein within the lens fiber cell membrane
	8. Yuka Koshiishi: Unilateral Pediatric Cataract with a Thinned Crystalline Lens: A Case Report
	9. Mayumi Nagata: A Case of Lamellar Separation of the Lens Capsule During Cataract Surgery
	10. Natsuko Hatsusaka: Cataract Survey in an Epidemiological Study on the Health Effects of Occupational Radiation
	11. Koichiro Mukai: Exploration of Hydrophilic Materials Less Prone to Calcium Deposition
	12. Kohei Miyata: Pupillary responses in eyes implanted with multifocal and monofocal intraocular lenses
	13. Norihiro Watanabe: Dynamic Pupillary Responses in Multifocal Intraocular lens (Synergy® and Odyssey®) Implanted Eyes
	14. Natsuko Maeda: Dynamic Pupillary Response and Accommodation with Intraocular Lenses and Crystalline Lenses
	15. Reona Mouri: Correlation with Lens accommodation parameters and Retinal age
	16. Yurui Pan: Cluster Analysis Identifying High-Risk Biometric Combinations for IOL Calculation Error in High Myopia
	17. Soyoka Goto: Postoperative Outcomes of Cataract Surgery with Intraoperative Mydriasis in Eyes with Primary Angles-closure
	18. Yuyan Shan: Pax6-K91R Mutant Mice Display Changed Transcriptome and Disrupted Lens Differentiation as Revealed by Single Cell RNA Sequencing (scRNA-seq) Study
	19. Pooja Rathaur: LOXL1 Loss Strengthens Ciliary Zonules and Alters Lens Epithelium Transcriptome
	20. Yu Du: ANGPTL7 Elevation Drives Intraocular Pressure Dysregulation in Highly Myopic Cataractous Eyes: A Multi-omics Insight
	21. Hirotaka Hashimoto: Effects of astaxanthin intake on adiponectin level in human aqueous humor
	22. Xue-Bin Hu: The Protein Phosphatase 1 Regulator, MYPT1 Control Lens Development through Direct Modulation of Functions of HMGA1 and Other Downstream Targets
	23. Jing-Miao Wang: Heat Shock Protein 90β Modulates Lens Inflammatory Response to Prevent Cataractogenesis via Direct Interaction with NF-κB p65
	24. Yuka Okada: Effects of Tenascin-X Deficiency on Anterior Segment Morphology
	25. Yosuke Nakazawa: Lens sclerosis is promoted by Piezo1 channel through transglutaminase 2 activation

Wednesday, December 10th	
8:30 am	**Session 8: Redox Biology & Cataract** *Julie Lim & Xingjun Fan*
	1. Xingjun Fan: Lipid Peroxidation Increases Lens Stiffness and Plasma Membrane αA-Crystallin Association
	2. Hongli Wu: Redox imbalance triggers lens inflammaging and senescence: insights from Grx1/Grx2 double-knockout mice
	3. Kim Jiang: Investigating the molecular mechanisms involved in cataract formation post vitrectomy
	4. Yu Pu: Removal of N-terminal cysteines from γD-crystallin generates aggregation-prone dimers
	5. Vincent Monnier: In silico methods for improving binding affinities of gamma crystallin aggregation inhibitors
	6. Julie Lim: Unlocking the clock: bringing to "light" the mechanisms involved in antioxidant regulation in the lens
10:00 am	*Break – Tea/Coffee*
10:30 am	**Session 9: Lens Optics & Biomechanics** *Barbara Pierscionek & Kehao Wang*
	1. Barbara Pierscionek: Reflections on the optics of the lens
	2. Ji He: Individual Variation of Refractive Index of the Crystalline Lens of the Normal and Cataract Eyes
	3. Qing Li: Retrieving the gradient refractive index structure of the crystalline lens from the wavefront aberrations over two-dimensional visual field
	4. Alyssa Lie: An in vivo MRI study of changes in lens water distribution with cataract development
	5. Kehao Wang: Biomechanical simulations reveal key parameters that affect lens accommodation
12:00 pm	***Afternoon Free / Golf Tournament***

Thursday, December 11th	
8:30 am	**Session 10: Lens Membranes & Physiology** *Tom White & Paul Donaldson*
	1. Jean Jiang: Dual-domain Regulation of Connexin 50 Suppresses Lens EMT and Post-surgical Fibrosis
	2. Taishi Painter: Role of Slc20a2 in Lens Growth and Fiber Cell Differentiation and Elongation
	3. Yadi Chen: Regional differences in Ca^{2+} mobilization mediated by zonular tension in the rat lens
	4. Nikhail Nair: Dissecting the contributions of AQPs to regional lens water homeostasis: insights from lens nanovesicle and hydrostatic pressure analysis
	5. Nick Delamere: A comparison of hydrostatic pressure responses to Piezo-1 and TRPV4 in the mouse lens
10:00 am	*Break – Tea/Coffee*
10:30 am	**Session 11: Lens Development & Regeneration** *Kelly Tseng & Mason Posner*
	1. Irene Vorontsova: Cross-talk between lens power and axial eye length: Unravelling mechanisms leading to myopia
	2. Hideaki Morishita: PLAAT phospholipases drive autophagy-independent organelle clearance in the lens for transparency
	3. Wei Li: PLCY functions in lens vesicle separation by modulating FGF signaling
	4. Kelly Tseng: Investigating Developmental Lens Regrowth
	5. Konstantinos Sousounis: Cellular states and trajectories during newt lens regeneration
12:00 pm	*Lunch*
1:15 pm	**Session 12: PCO, Fibrosis and EMT** *Janice Walker & Michael Wormstone*
	1. Janice Walker: Defining the metabolic and bioenergetic requirements that fuel the lens response to injury to promote a fibrotic outcome to wounding
	2. Promita Deb: Role of α5β1 integrin in the pathogenesis of fibrotic posterior capsular opacification (PCO)
	3. Lan Zhang: CREB controls STING-Mediated Signaling of Inflammatory Response of the Ocular Lens through the Transcriptional Repressor REST and STING ISGylation during Lens Fibrosis
	4. Qiuli Fu: Generation and application perspectives of regenerated lens during cataract treatment
	5. Michael Wormstone: Menadione-induced depletion of glutathione (GSH) impairs lens epithelial cell function and viability: A potential strategy for posterior capsule opacification management
2:45 pm	*Break – Tea/Coffee*

Thursday, December 11th	
3:00 pm	**Session 13: Crystalline Lens Optical Evaluation; Inspiration and Guidance for IOLs and Presbyopia Treatment** *Susana Marcos*
	1. Susana Marcos: Crystalline lens full 3-D geometry: implications for intraocular lens development and selection
	2. Kirill Larin: Optical Coherence Elastography and Brillouin Spectroscopy of Crystalline Lens
	3. Liane Clamen: Development of an Accommodating Intraocular Lens that Mimics the Young, Human Crystalline Lens
	4. Andres de la Hoz: Finite element models of accommodation for estimation of crystalline lens material properties
	5. Alberto de Castro: Optical coherence tomography to study the gradient index of refraction of the human crystalline lens
	6. Eduardo Martinez-Enriquez: Eigenlens geometrical representation of the crystalline Lens
4:30 pm	*Break*
5:30 pm	**The Kinoshita Lecture**
6:30 pm - 9:00 pm	***Conference Banquet at Bayview Grounds***

Friday, December 12th	
8:30 am	**Session 14: Molecular Aging in Lens and Non-Lens Tissues** *Kevin Schey & Christina Karakosta*
	1. Christina Karakosta: Comparative Proteomic Analysis of Aqueous Humour, Anterior Lens Capsules, and Crystalline Lenses in Different Human Cataract Subtypes versus Healthy Controls
	2. Sarah Zelle: Analysis of adult Nrf2 and αB-crystallin mutant zebrafish lenses exposed to oxidative stress reveals shifts in the lens proteome
	3. Preston Hazen: N101D Deamidation and Cholesterol Content Differentially Impact the Membrane Binding of αA-Crystallin to Model Human Lens Lipid Membranes
	4. Kevin Schey: Molecular Aging in the Lens: What are We Missing?
	5. Lee Goldstein: Molecular Aging and Diabetic Protein Glycation Detected and Tracked In Vivo by Quasi-Elastic Light Scattering Spectroscopy Scanning of the Lens
10:00 am	*Break – Tea/Coffee*

Friday, December 12th	
10:30 am	**Session 15: No Lens is an Island** *Steve Bassnett & Katelyn Swindle-Reilly*
	1. Katelyn Swindle-Reilly: The Lens Is Not an Island: Strategies from the Vitreous to Support Lens Health
	2. Jodi DeDreu: How the lens functions in regulating immune responses to maintain/restore homeostasis in the eye
	3. Marco Ruggeri: Quantifying the Ciliary Muscle in Accommodation with Optical Coherence Tomography
	4. Juan Rodriguez: Tweezing out zonular mechanics
	5. Steve Bassnett: Developing an Anatomically Accurate Base Model of the Human Eye with Emphasis on the Zonular Apparatus
12:00 pm	**Closing Remarks and Depart**


Sunday, December 7th

Plenary Lecture

Intracellular degradation by autophagy and PLAAT family lipases

Noboru Mizushima[1]

[1]*Department of Biochemistry and Molecular Biology, Graduate School of Medicine, The University of Tokyo, Tokyo, Japan*

Autophagy is an intracellular degradation system conserved in many eukaryotes. In the process of autophagy, a portion of the cytoplasm is surrounded by autophagosomes and then delivered to lysosomes for degradation. Studies on the molecular mechanism and physiological function of autophagy have made remarkable progress over the past 30 years since the landmark genetic studies in yeast by Dr. Ohsumi and other groups.

In this lecture, I will first review the molecular mechanism and physiological functions of autophagy in mammals and then talk about a recent topic on the functional recovery of the nervous system using a novel mouse model in which autophagic activity can be rapidly and reversibly regulated in a doxycycline-dependent manner. Suppressing autophagy led to proteome and transcriptome changes, inclusion body accumulation, and axonal swelling, all of which were largely ameliorated after autophagy restoration. Consistent with these cellular abnormalities, autophagy suppression caused motor and cognitive dysfunction in mice, which was also significantly reversed upon autophagy restoration. These results suggest that neurological dysfunctions caused by autophagy defects can be rescued by autophagy restoration and provide proof-of-concept for therapeutic targeting of autophagy in neurodegenerative diseases.

In the second part, I will show our recent work on autophagy-independent organelle degradation in the lens. In eye lenses, cells undergo massive degradation of all organelles during terminal differentiation even in autophagy-deficient mice. Using zebrafish and mice, we revealed that PLAAT (phospholipase A and acyltransferase) family phospholipases are essential for the degradation of lens organelles such as the ER, mitochondria, and lysosomes. PLAATs translocate from the cytosol to these organelles to degrade them. This new type of organelle degradation is essential for achieving optimal transparency and refractive function of the lens. Thus, various mechanisms exist for organelle degradation.

Monday, December 8th

Monday, December 8th

Session 1: Lens Cell and Matrix Biology

Moderators: *Cathy Cheng & TJ Plageman*

Talks are in the format of 15 minutes presentation with 3-minute discussion.

TIME	SPEAKER	TITLE
8:30 am	**TJ Plageman**	Arvcf-dependent cell adhesion at lens sutures
8:48 am	**Justin Parreno**	F-actin reorganization as a regulator of epithelial-mesenchymal transition in a serum/growth-factor free ex vivo model of lens fibrosis
9:06 am	**Juliet Moncaster**	Microtubule-Associated Protein Tau (MAPT) in mouse non-transgenic and transgenic Alzheimer's Disease lenses
9:24 am	**Matthew A. Reilly**	The Lens' Gradient Refractive Index Deteriorates Upon Removal of the Capsule and Epithelium
9:42 am	**Cathy Cheng**	Eph-ephrin signaling affects lens growth and shape, nucleus size, and gradient refractive index in adult mice

Arvcf-dependent cell adhesion at lens sutures

Timothy F. Plageman Jr.[1], Anna Lubertozzi[1], and Electra Coffman[1]

[1]*The Ohio State University College of Optometry*

Purpose: Lens sutures form where the apical and basal tips of lens fiber cells meet creating a complex three-dimensional seam along the anterior-posterior axis of the lens. Recent evidence has emerged suggesting that this structure is enriched in the adherens junctional proteins N-cadherin and Arvcf. It is hypothesized that cadherin-associated adhesion is required for the integrity of the lens suture and when disrupted may induce lens opacities.

Methods: Mice harboring the Wnt1-cre driver and ROSAmT/mG; transgenes were generated to analyze genetically labeled fluorescent lens fiber cells. Arvcf deficient lenses were also generated in mice using a genetic knock-out allele. Whole lenses were isolated and examined via static and live imaging utilizing a stereo and confocal microscopy. Histological sections of lens tissue were also immunofluorescently labeled and analyzed.

Results: Upon analysis of Wnt1-cre; ROSAmT/mG lenses, it was serendipitously observed that sporadic cre-reporter activity occurs in the lens. When the sutural region of these lenses was analyzed, a complex architecture of individual lens fiber cell tips were discovered hinting at the requirement for a large surface area. Cell adhesion proteins such as N-cadherin and Arvcf are strongly localized to the lens sutural region. To test if lens fiber cell adhesion is required for lens sutural integrity, Arvcf deficient animals were observed. Absence of Arvcf causes separation of the sutures with age and eventual volcano-like blowouts causing anterior polar lens opacities. The sutural defects precede that of the cortical opacities in Arvcf deficient animals suggesting that sutural disruption may be a causative factor.

Conclusions: Lens fiber cell sutures, like cortical transparency maintenance, require Arvcf/N-cadherin-based cell adhesion to maintain normal lens architecture and transparency. Cortical cataract development could therefore be partially caused by disturbances to the adhesive ability of fiber cells at lens sutures.

F-actin reorganization as a regulator of epithelial-mesenchymal transition in a serum/growth-factor free ex vivo model of lens fibrosis

Justin Parreno[1, 2], Rylee E. King[1]

[1]*Department of Biological Sciences, University of Delaware, Newark, DE, USA*
[2]*Department of Biomedical Engineering, University of Delaware, Newark, DE, USA*

Purpose: Lens epithelial-to-myofibroblast transition (EMT) drives lens fibrosis in various types of cataracts. To gain insight into the sequalae of lens EMT, we developed a novel ex vivo whole lens culture model that initiates EMT without exogenous TGFβ. We investigated the temporal alterations in cell morphology, cytoskeleton, and molecular expression. Using this model, we examine the regulation of EMT by F-actin reorganization and F-actin associated proteins.

Methods: Freshly isolated mouse lenses were cultured in serum- and growth factor-free medium. At defined time points, we analyzed cell morphology, F-actin organization, EMT marker expression (Col1, Tnc, Fn, αSMA), and the association of stress fiber–associated proteins Tropomyosin 3.1 (TPM3.1) and Transgelin (TAGLN) with F-actin.

Results: We found that within 4 hours of culture, lens epithelial cells reorganized F-actin into stress fibers at the basal region of lens epithelial cells. The proportion of basal F-actin stress fibers increased with time. Concomitantly, F-actin disassociated from the cortex at the lateral and apical region of the epithelial cells. This F-actin reorganization preceded the upregulation of the fibrotic molecules Col1, Tnc, Fn, and asma mRNA levels which occurred after 24 hours of culture. We also found that TPM3.1 and TAGLN upregulate early in EMT and associate with F-actin in stress fibers.

Conclusions: Our ex vivo model reveals that lens EMT can be initiated in the absence of exogenous TGFβ. Since F-actin reorganization occurs early in EMT, targeting critical regulators of F-actin reorganization into stress fiber could lead to new therapeutic insight against EMT related cataracts.

Microtubule-Associated Protein Tau (MAPT) in mouse non-transgenic and transgenic Alzheimer's Disease lenses

Juliet A. Moncaster[1,2], Paulina Aragay Herrera Blanc[1], Elijah Demb[1], Olga Minaeva[1,2], Lee E. Goldstein[1,2]

[1]*Department of Radiology, Boston University Chobanian & Avedisian School of Medicine, Boston, MA, USA*
[2]*Boston University Alzheimer's Disease Research Center, Boston, MA, USA*

Purpose: We previously discovered that Aβ accumulates in the cortical/supranuclear region of the lens in people with Alzheimer's Disease (AD) (Goldstein et al., 2003) and Down Syndrome (DS; (Moncaster et al., 2010). We also demonstrated Aβ in the Tg2576 APP Swedish mutation AD mouse model (Moncaster et al., 2022). Another protein that is involved in AD is Microtubule- Associated Protein Tau (MAPT, Tau). Under normal conditions, tau plays a role in microtubule stabilization in the brain. However, in AD tau becomes hyperphosphorylated which results in microtubules destabilization and eventual formation of neurofibrillary tangles. Tau has previously been reported to be expressed in the mouse lens (Bai et al., 2007; Zhao et al., 2013) but the results vary depending on the mouse model and antibodies used. Here we investigated whether tau protein was expressed in non-transgenic mouse lenses and in a transgenic tau mutant (P301S) and whether there was a cataract phenotype.

Methods: P301S tau mutant transgenic and non-transgenic mice were bred and maintained at Boston University. Breeder mice were purchased from Jackson Laboratory. Male and Female transgenic and non-transgenic mice were sacrificed throughout their lifespan at ages 3-12 months. Mice were perfused with phosphate buffer saline; lenses were isolated and then imaged under two different sources of light using a D70 digital Nikon camera and a custom-adapted Zeiss stereophotomicroscope. Lenses were then snap frozen and analyzed by Western Blotting (1 lens per lane) using a panel of tau antibodies.

Results: P301S transgenic and non-transgenic mouse lenses all expressed tau during their lifespan when analyzed by Western blot. P301S transgenic lenses demonstrated an additional higher molecular weight band compared to the non-transgenic mice. No cataract phenotype was observed in transgenic or non-transgenic mouse lenses.

Conclusions: There was no overt difference in lens phenotype between the P301S transgenic and non-transgenic mice. However, the banding pattern observed by Western blot for P301S transgenic mice compared to non-transgenic was different. Our data suggests the P301S tau mutation affects tau processing but does not result in cataract phenotype. Based on our current and previous results, the data suggests that Aβ may play a more significant role than tau in lens pathology in AD.

The Lens' Gradient Refractive Index Deteriorates Upon Removal of the Capsule and Epithelium

Haneet Kang[1], Myles Cline[2], Zachery Moser[2], Matthew A. Reilly[2,3]

[1]*Department of Mechanical and Aerospace Engineering, The Ohio State University, Columbus, OH, USA*
[2]*Department of Biomedical Engineering, The Ohio State University, Columbus, OH, USA*
[3]*Department of Ophthalmology & Visual Science, The Ohio State University, Columbus, OH, USA*

Purpose: We recently proposed a model wherein the lens capsule provides tension to counterbalance the pressure of fiber cell compaction. Here, we experimentally test this theory by measuring the evolution of the gradient refractive index (GRIN) in the presence and absence of the lens capsule.

Methods: Young, fresh porcine lenses were carefully dissected. One lens from each pair remained encapsulated, while its fellow lens was carefully decapsulated. Both lenses were then placed side by side in a custom chamber for photogrammetry and evaluation using a scanning laser to measure the refractive properties of the lens. The chamber was filled with phosphate-buffered saline and maintained at 34°C throughout the 24-hour testing period.

Results: Decapsulated lenses swelled significantly over a period of hours, despite submersion in a nominally physiological saline, whereas encapsulated lenses retained a nearly constant geometry over the period of observation. The GRIN deteriorated in the decapsulated lenses, whereas it was largely retained in the encapsulated lenses.

Conclusions: These findings suggest that the presence of the lens capsule, and/or its adherent epithelium, are required to maintain the GRIN by dehydrating the central portion of the lens. This empirical observation suggests that biomechanical compaction forces may be applied by the lens capsule and/or the nucleus may be actively dehydrated via the lens microcirculation. Additional experiments are required to discern the relative contributions of these two explanations.

Eph-ephrin signaling affects lens growth and shape, nucleus size, and gradient refractive index in adult mice

Catherine Cheng[1], Gryffin M. Flowers[1], Kehao Wang[2], Masato Hoshino[3], Kentaro Uesugi[3], Naoto Yagi[3], Barbara Pierscionek[4]

[1]School of Optometry and Vision Science Program, Indiana University, Bloomington, IN, USA
[2]Beijing Advanced Innovation Center for Biomedical Engineering, School of Engineering Medicine, Beihang University, Beijing, China
[3]Japan Synchrotron Radiation Research Institute (SPring-8), 1-1-1, Kouto, Sayo-cho, Sayo-gun, Hyogo, Japan
[4]Faculty of Health, Medicine and Social Care, Medical Technology Research Centre, Anglia Ruskin University, Chelmsford, United Kingdom

Purpose: The function of the eye lens to fine focus light from different distances onto the retina
to form a clear image relies on tissue biomechanical properties, refractive index, shape, and transparency. Increased lens stiffness with age, especially of the center or nucleus, has long been hypothesized to lead to presbyopia. The cellular and molecular mechanisms that determine lens
biomechanical properties and change during age-related stiffening remain unclear.

Methods: We investigate whether lens stiffness, resilience, morphometric, or gradient refractive index (GRIN) changes occur in lenses from 4- and 8-month-old adult mice with disruption of Eph-ephrin signaling.

Results: We previously showed that loss of EphA2, a receptor tyrosine kinase, or ephrin-A5, a ligand for Eph receptors, leads to changes in lens shape and resilience in 2-month-old mice. Surprisingly, the loss of EphA2 led to smaller and softer lens nuclei with no change in lens stiffness. Our new data revealed no obvious changes in lens stiffness or resilience between control and ephrin-A5 knockout (KO or -/-) mice at 4 and 8 months of age. While there were no differences in lens resilience, EphA2-/- lenses were stiffer than control lenses from 8-month-old mice. At all ages, EphA2 and ephrin-A5 KO lenses were more spherical in shape, and EphA2-/-lens nuclei were smaller than controls. In 4- and 8-month-old mice, EphA2-/- lenses were small. Measurement of GRIN in control and KO lenses revealed that EphA2-/- lenses had decreased magnitudes of refractive index across the GRIN profile in all age groups.

Conclusions: These results suggest that, at least in mouse lenses, the size of the lens and nucleus does not affect whole tissue stiffness with age. Our work indicates that Eph-ephrin signaling influences lens shape and normal adult whole lens growth while EphA2 is needed for nuclear size and appropriate GRIN.

Monday, December 8th

Session 2: Lens Differentiation

Moderators: *Amer Riazuddin & Salil Lachke*
Talks are in the format of 15 minutes presentation with 3-minute discussion.

TIME	Speaker	Title
10:30 am	**Amer Riazuddin**	The role of autophagy in lens fiber cell differentiation
10:48 am	**Michael L. Robinson**	FOXE3 transcriptional targets suggest a role for FOXE3 in suppressing neural differentiation
11:06 am	**Jared Tangeman**	Leveraging single-cell multiomics to resolve gene regulatory networks governing lens fiber cell differentiation
11:24 am	**Velia Fowler**	Delayed Fiber Cell Maturation in CAP2cKO Mouse Lenses
11:42 am	**Salil Lachke**	Ell2 is necessary for optimally accessible chromatin in early differentiation stages to achieve high levels of select transcripts in fiber cells

The role of autophagy in lens fiber cell differentiation

S. Amer Riazuddin[1], Shahid Y. Khan[1], Muhammad Ali[1], Firoz Kabir[1], Hira Butt[1], and J. Fielding Hejtmancik[2]

[1]The Wilmer Eye Institute, Johns Hopkins University School of Medicine, Baltimore, MD
[2]National Eye Institute, National Institutes of Health, Bethesda, MD

Purpose: The lens has a unique need for the removal of all cellular organelles present in differentiating fiber cells to maintain transparency, an adaptation not essential for the physiological functioning of other cells in the eye. We previously reported cataract-causing mutations in FYCO1, an autophagy-associated gene. We recently demonstrated that FYCO1-dependent autophagy is crucial for lens transparency, operates independently of basal autophagy, and supports lens fiber cell differentiation. The purpose of this study is to further elucidate the role of autophagy in lens fiber cell differentiation.

Methods: We employed immunohistochemistry, immunocytochemistry, western blot, and flow cytometry analyses to examine knock-in mouse lens and knock-in human lens epithelial cells.

Results: Our results show that deficiency of FYCO1 causes autophagosomes to accumulate near the perinuclear area with diminished autophagy flux in lens cells. Phosphorylation of LC3B at Thr50 is reported to trigger the release of LC3B from FYCO1, and in line with published studies, we found that Thr50Ala substitution in LC3B results in accumulation of autophagosome near the cell periphery with a decrease in the autophagic flux in lens cells. Additionally, shRNA-mediated knockdown of adaptor proteins linked to dynein-dynactin also resulted in accumulation of autophagosomes near the cell periphery with diminished autophagy in lens cells.

Conclusions: Our data, taken together with results published from other prominent laboratories, suggest that the differentiating lens fiber cells need FYCO1-dependent autophagy to achieve complete organelle removal. According to our model, autophagosomes attached to FYCO1 are driven by kinesin towards the cell periphery to engulf organelles residing far from the perinuclear region. The autophagosomes detach from FYCO1 at or near the cell periphery upon phosphorylation of LC3B at Thr50, bringing an end to the kinesin-dependent transport of autophagosomes. These autophagosomes are then transported back to the perinuclear area by dynein-dynactin to fuse with lysosomes for organelle degradation.

FOXE3 transcriptional targets suggest a role for FOXE3 in suppressing neural differentiation

Michael L. Robinson[1], Anil Upreti[1], Jared A Tangeman[1], Jacob M Weaver[1], Nour Sayed[1]

[1]Department of Biology, Center for Visual Sciences, Miami University, Oxford, OH, USA

Purpose: The transcription factor, FOXE3, is required for normal lens development. However, the precise mechanism by which FOXE3 supports lens development remains unknown. To understand FOXE3 function, we have sought to identify FOXE3 transcriptional targets by combining unbiased sequence-based approaches and bioinformatics tools.

Methods: CRISPR/Cas9 genome editing was used to create mutations at the Foxe3 locus. Lens RNA was collected from wild-type and Foxe3 null lenses at E12.5, E15.5 and P0 and used for bulk RNA-sequencing. Differentially expressed genes at all three developmental timepoints were subjected to gene ontology (GO) analysis. Wild-type lens epithelial cells at P0 were used for CUT&RUN analysis utilizing antibodies to FOXE3, H3K4me3 and H3K27ac. FOXE3 transcriptional targets were selected based on two major criteria: 1) they exhibit differential expression in Foxe3 null lenses and 2) they have a FOXE3 CUT&RUN-verified binding site in their promoter and/or enhancer regions.

Results: *Foxe3* null lenses differentially expressed 376, 1,239 and 2,553 genes relative to wild-type lenses (Log2 Fold Change >1. pAdjust <0.005) at E12.5, E15.5 and P0, respectively. When comparing across all timepoints, more genes are upregulated than downregulated in the Foxe3 null lenses. GO analysis found “lens development in camera-type eye” and “nervous system development” as the top terms in the downregulated and upregulated genes, respectively. CUT&RUN identified 1,507 genomic FOXE3 binding sites commonly identified by two different FOXE3 antibodies in lens chromatin. These binding sites identified an enriched DNA sequence motif most closely matched with the known FOXD3 binding motif. These peaks included genes associated with lens differentiation (*Bfsp1, Mafb* and *Pdgfra*) and neural development (*Sox4, Rorb, Sox11*, and *Sema6c*).

Conclusions: The loss of FOXE3 during lens development leads to a progressive deregulation of gene expression that includes the down-regulation of genes associated with lens development and upregulation of genes associated with neural differentiation.

Leveraging single-cell multiomics to resolve gene regulatory networks governing lens fiber cell differentiation

Jared A Tangeman[1], Sanjaya K. Shrestha[3,4], Salil A. Lachke[3,4], Seth Blackshaw[1,2]

[1]*Solomon H. Snyder Department of Neuroscience, The Johns Hopkins University School of Medicine, Baltimore, MD, USA*
[2]*Department of Ophthalmology, The Johns Hopkins University School of Medicine, Baltimore, MD, USA*
[3]*Department of Biological Sciences, University of Delaware, Newark, DE, USA*
[4]*Center for Bioinformatics and Computational Biology, University of Delaware, Newark, DE, USA*

Purpose: Single-cell (sc) multiomics enables simultaneous chromatin and transcriptome profiling of individual cells, opening the door to understanding how transcription factor-chromatin interactions drive cellular differentiation. Previous studies have applied scRNA/ATAC-seq to profile transcriptionally distinct transition states during fiber cell differentiation. To expand on these perspectives, we sought to apply integrated multiomic analyses to lenses across a broad range of mouse developmental stages, with the objective of reconstructing gene regulatory networks driving lens development.

Methods: Combined scRNA/ATAC-seq was performed on the embryonic mouse lens, encompassing embryonic day 10.5 (E10.5) through E17.5, as well as postnatal day 10 and 15 lenses. Gene expression data was used to identify genes with dynamic expression during fiber cell differentiation. Key genes were spatially resolved using in situ hybridization. Multiomic data were used for in silico gene regulatory network prediction using the SCENIC+ analysis pipeline.

Results: Combined scRNA/ATAC profiling resolved embryonic mouse lens cell clusters associated with epithelial, intermediate differentiating, and fiber cell populations, with a total of 1277 differentially expressed genes. Comparable populations were identified in the postnatal lens, with 4837 total differentially expressed genes. 1082 genes were associated with the intermediate cell cluster in the postnatal lens, largely enriched for cytoskeletal elements and cell morphogenesis. In situ hybridization of novel intermediate genes markers, such as Ptpru and Nhs, confirmed localization of these genes to transitioning cells at the lens fulcrum. Gene regulatory network reconstruction resolved downstream gene targets for dozens of transcription factors, including known regulators Maf and Hif1a, as well as contextually novel factors, such as Nfia and Nfib.

Conclusions: Single-cell multiomics provides a powerful platform for the discovery of dynamically expressed genes during fiber cell differentiation. Multiomic analysis enables the prediction of gene regulatory networks active in lens development and can elucidate potential functions of these regulators in fiber cell differentiation.

Delayed Fiber Cell Maturation in CAP2^{cKO} Mouse Lenses

Velia M. Fowler[1], Sepideh Cheheltani[1]

[1]Department of Biological Sciences, University of Delaware, Newark, DE, USA

Purpose: Actin cytoskeleton remodeling is essential for proper differentiation and maturation of lens fiber cells, supporting lens biomechanics and transparency. Lens fiber cells undergo distinctive morphological and molecular changes during differentiation and maturation, including formation of membrane paddles and protrusions, followed by membrane ridges and grooves in inner fiber cells, a hallmark of fiber cell aging. In previous work, we showed that altered levels of select actin-binding proteins affected lens paddles and protrusions and lens stiffness. Here, we investigated the role of Cyclase-associated actin cytoskeleton regulatory protein 2 (CAP2), a conserved actin-binding protein, in lens fiber cell maturation and age-associated morphological changes.

Methods: Using a lens-specific CAP2 conditional knockout (CAP2^{cKO}) mouse, we employed immunostaining, imaging, and Scanning Electron Microscopy (SEM) to identify the role of CAP2 in fiber cell maturation and lens aging.

Results: SEM imaging revealed that membrane ridge and groove formation initiated at ~320 µm from the surface in control lenses but showed a marked delay in CAP2^{cKO} lenses, with membrane ridges appearing at depths > ~530 µm. Consistent with delayed maturation, immunostaining of CAP2^{cKO} lenses revealed the persistence of full-length AQP0 in the deeper fiber layers, where it is typically truncated in controls. These findings suggest altered cytoskeletal regulation and delayed AQP0 processing in the absence of CAP2, potentially mediated by increased levels of the membrane-actin linker protein ezrin, which we also observed. Strikingly, aged CAP2^{cKO} lenses (40 weeks old) exhibited a significantly enlarged lens nucleus, the hardened fiber cell core that accumulates with age.

Conclusions: These data suggest that CAP2, by regulating the timing of fiber cell maturation, may limit the extent of fiber cell compaction in the nucleus of aged mice. Together, our findings support a critical role for CAP2 in coordinating cytoskeletal remodeling, membrane maturation, and the gross morphology of the aging lens.

Ell2 is necessary for optimally accessible chromatin in early differentiation stages to achieve high levels of select transcripts in fiber cells

Salil A. Lachke[1,2], Sanjaya K. Shrestha[1], Jared A. Tangeman[3]

[1]Department of Biological Sciences, University of Delaware, Newark, DE, USA
[2]Center for Bioinformatics and Computational Biology, University of Delaware, Newark, DE, USA
[3]Solomon H. Snyder Department of Neuroscience, The Johns Hopkins University School of Medicine, Baltimore, MD, USA

Purpose: For the past 50 years, the lens has been studied as an extreme example of cell type-specific gene expression. Fiber cells massively upregulate expression of key mRNAs while progressing toward organelle-loss and thus have the added temporal challenge of achieving the proper mRNA dosage prior to de-nucleation and irreversible loss of transcription potential. Our understanding of how lens cells achieve this striking upregulation of fiber-expressed mRNAs (e.g. crystallins, aquaporins, etc.) represents a significant knowledge-gap, which is addressed here.

Methods: iSyTE, RT-PCR, in situ hybridization, Western, and immunostaining were used to examine expression. Pax6GFPCre was used to generate Ell2 lens-conditional knockout mice ($Ell2^{cKO}$). Single-nucleus (sn) multiomics (snRNA-seq+snATAC-seq) were performed.

Results: We applied iSyTE to predict Ell2 (Elongation factor for RNA polymerase II 2), implicated in general transcription elongation, mediates selective gene expression control in lens development. Ell2 lens expression is conserved in vertebrates, including humans. Ell2cKO mice develop early-onset lens defects and exhibit reduced expression of select genes (12 crystallins, 2 aquaporins, etc.) linked to human/animal cataract. snATAC-seq shows reduced chromatin accessibility across crystallin promoter regions (e.g., Cryge, etc.) specifically in early, but not late, fibers in $Ell2^{cKO}$ lens. These new data support an exciting model wherein Ell2 is necessary for optimally-open chromatin over key lens genes early in fiber differentiation for achieving high mRNA levels when there are optimal levels of the TFs Maf, Pax6, Prox1, Sox1.

Conclusions: A long-standing question in lens research is whether the present knowledge of the lens transcription factors is sufficient to explain high-level gene expression in fiber cells. Our findings address this critical knowledge-gap by showing that Ell2 is essential for open chromatin at select loci for building up high levels of transcripts in lens fibers. Further, these data suggest that components of the general transcription process can play a selective role in developing tissues.

Monday, December 8th

Session 3: Structural Biology and Biophysics

Moderators: *Rachel Martin & Tamir Gonen*

Talks are in the format of 15 minutes presentation with 3 minutes discussion.

Time	Speaker	Title
1:15 pm	**Rachel Martin**	Biophysical characterization of site-specifically oxidized lens proteins
1: 33 pm	**Shane Gonen**	CryoEM imaging methodology for difficult protein targets
1:51 pm	**Rachel Klevit**	Parsing the grammar of small heat shock proteins
2:09 pm	**Tamir Gonen**	MicroED solution to the lens MP20 enigma
2:27 pm	**Haruka Matsumoto**	Analysis of cataract pathophysiology analysis at the molecular level using mass spectrometry imaging

Biophysical characterization of site-specifically oxidized lens proteins

Rachel W. Martin[1,2], Catherine Seo[1], Collin Sroge[2], Zane G. Long[1]

[1]Department of Chemistry, University of California, Irvine, CA, USA
[2]Center for Bioinformatics and Computational Biology, University of California, Irvine, CA, USA

Purpose: The human eye lens consists of densely packed crystallin proteins that exhibit remarkable solubility despite minimal protein turnover. Post-translational damages that accrue over a lifetime can reduce crystallin solubility, resulting in the precipitation or phase-separation of protein aggregates. Oxidation is a common type of modification that can cause such opacification of the lens, particularly in age-related cataracts. Despite its importance, oxidation is difficult to study using biophysical methods due to the difficulty of preparing homogenous samples. Here we use genetic code expansion (GCE) to introduce site-specific modifications mimicking the damage caused by oxidation.

Methods: Oxidized crystallin proteins were produced using recombinant expression in E. coli and GCE. Biophysical characterization was performed using NMR spectroscopy, mass spectrometry, and optical spectroscopy.

Results: We incorporated a model oxidation product, 5-hydroxytryptophan (5-HTP), at residue 163 of γS-crystallin using a GCE platform. The resulting oxidized variant was characterized using optical spectroscopy. Although it was similar to wild-type under ambient conditions, the oxidized variant showed diminished resilience against thermal and chemical stress. The underlying conformational changes were investigated using NMR spectroscopy and were found to be localized near the oxidized residue; the disruption is based on subtle local changes rather than global unfolding.

Conclusions: These data demonstrate that a common post-translational modification observed in human γS-crystallin increases its aggregation propensity to the extent that aggregation is observed near physiological temperature. Furthermore, our findings highlight the utility of GCE platforms for systematically evaluating site-specific post-translational modifications and provide insight into the molecular mechanisms of age-related cataracts.

CryoEM imaging methodology for difficult protein targets

Shane Gonen[1]

[1]*University of California, Irvine, Irvine, CA, USA*

As conduits between the outside world and the inner cellular environment, membrane proteins play important roles in our bodies, including those that go awry and cause disease. Despite recent technological advances, structural studies of these proteins still present many challenges. We develop methodology and use cryogenic electron microscopy (CryoEM) to study human membrane proteins, including ion channels and occular G protein-coupled receptors. I will describe some of our recent efforts in using both new grid developments and fiducial markers to reach high-resolution. These techniques allowed us to bypass biochemical challenges and size limitations of CryoEM.

Parsing the grammar of small heat shock proteins

Rachel E. Klevit[1], Maria K. Janowska[1], Christopher N. Woods[1], Lindsey D. Ulmer[2], Natalie L. Stone[1], Miklos Guttman[3], Matthew F. Bush[2]

[1]Department of Biochemistry, University of Washington School of Medicine, Seattle, WA, USA
[2]Department of Chemistry, University of Washington, Seattle, WA, USA
[3]Department of Medicinal Chemistry, University of Washington, Seattle, WA, USA

Purpose: Small heat-shock proteins (sHSPs) are a widely expressed family of ATP-independent chaperones that delay protein aggregation during stress and under basal conditions. Three sHSPs—HSPB1 (Hsp27), HSPB4 (αA-crystallin), and HSPB5 (αB-crystallin)—are central to eye lens development and lifelong maintenance. All three form dynamic, polydisperse oligomers enriched in disorder, long resistant to structural analysis. We developed approaches that reveal critical information on the challenging disordered regions. Our goal is to pinpoint regions responsible for client-specific chaperone activity.

Methods: We applied hydrogen–deuterium exchange mass spectrometry (HDX-MS) and photo-crosslinking to native oligomers of HSPB1, HSPB4, and HSPB5. We applied hydrogen–deuterium exchange mass spectrometry (HDX-MS) and photo-crosslinking to native oligomers of HSPB1, HSPB4, and HSPB5. Comparisons included wild-type proteins, disease mutants causing early cataract, phosphorylated variants, and chimeric proteins between family members.

Results: HSPB4 and HSPB5 show distinct activity toward the lens client γD-crystallin. Swapping as few as three residues within the N-terminal region (NTR) switches their activity profiles. This NTR segment regulates exposure of a “Long Hydrophobic” region. Pathogenic HSPB5 mutants R120G and D109H, as well as a phosphomimic at one phosphorylation site, display increased exposure of this region, all correlating with enhanced chaperone activity toward γD-crystallin.

Conclusions: Rather than simple disordered stretches toggling between “all in” or “all out,” sHSP NTRs comprise a network of short interactive motifs responsive to conditions, modifications, and mutations. In HSPB5, sequestration versus exposure of the Long Hydrophobic region within the ~60-residue NTR largely determines its ability to maintain damaged γD-crystallin in soluble form. These insights raise the possibility of targeting specific sHSP functions with small molecules or related strategies.

MicroED solution to the lens MP20 enigma

Tamir Gonen[1]

[1]*Department of Biological Chemistry, University of California, Los Angeles, CA, USA*

My laboratory studies the structures of membrane proteins that are important in maintaining cellular homeostasis. Understanding structure requires an atomic structural model of the protein of interest captured in various functional states. We developed the method Microcrystal Electron Diffraction (MicroED) and demonstrated that it is feasible to determine protein structures by electron diffraction using vanishingly small crystals. I will describe our efforts in the MicroED field and illustrate how this technique allows us to determine structures for novel proteins that were beyond the reach of other methods. I will use the small membrane protein MP20 from the eye lens as a case study and reveal an unexpected role for this protein as a tight junction protein.

Analysis of cataract pathophysiology analysis at the molecular level using mass spectrometry imaging

Haruka Matsumoto[1], Satoshi Ogawa[1], Hiroyuki Matsushima[1], Mayumi Nagata1, Tatsutaka Kawagoe[1]

[1]Department of Ophthalmology, Dokkyo Medical University Hospital, Tochigi, Japan

Purpose: Despite various changes to the lens tissue being reported during cataract progression, the underlying molecular mechanisms remain unclear. The aim of this study was to analyse localisations and qualitative variations in constituent molecules by collecting lenses or lens capsules from cases with various grades (Emery-Little classification) and types of cataracts.

Methods: With the appropriate ethical approval (Dokkyo Medical University Hospital R-81-7J) in place, lens capsules and tissues were collected from the eyes of 40 cataract patients undergoing surgery. These patients had cataracts that were either age-related (28 eyes), atopic (five eyes), traumatic (three eyes) or congenital (four eyes). Phacoemulsification with continuous curvilinear capsulorhexis (CCC) was performed on 36 eyes, while intracapsular cataract extraction (ICCE) was performed on four eyes. The lens and lens capsules were frozen-embedded and sectioned for histopathological analysis using haematoxylin and eosin (HE) staining, methenamine (MT) staining and anti-crystallin antibody staining. Only the lens capsules were stained with trypan blue. The adjacent sections underwent mass spectrometry imaging analysis using an iMScope (Shimadzu), after a matrix was applied to promote ionisation of the molecules.

Results: Various substances, including cholesterol and flavin mononucleotide, were found to be distributed differently throughout the lens nucleus, cortex and entire lens. Although it has been reported that FMN increases with cataract progression, this study suggests that its localisation changes in ICCE cases. The quantity and localisation of FMN appeared to change with cataract progression. Principal component analysis also indicated the presence of components that could differentiate between levels of cataract nucleus hardness.

Conclusions: Changes in molecular localisation could be used as potential biomarkers for patient stratification and cataract prevention.

Monday, December 8th

Session 4: Cataract Genetics

Moderators: *Elena Semina & Anne Slavotinek*

Talks are in the format of 15 minutes with 3 minutes discussion.

Time	Speaker	Title
3:00 pm	**Elena Semina**	Uncovering new genetic factors in pediatric cataracts
3:18 pm	**Gavin Arno**	Nanopore sequencing transcript analysis and functional investigation of a novel CYP51A1 genotype confirms the gene's association with autosomal recessive congenital cataract
3:36 pm	**Hélène Choquet**	Novel cataract risk loci identified from a large-scale multiethnic genome-wide association meta-analysis
3:54 pm	**Fielding Hejtmancik**	Aspartyl (Asparaginyl) beta-Hydroxylase deficiency impairs lens placode invagination and lens development in a Traboulsi syndrome Mouse Model
4:12 pm	**Anne Slavotinek**	Single cell RNA-Seq in a zebrafish model of crim1 loss of function – uncovering gene regulatory networks and mechanisms for lens development

Uncovering new genetic factors in pediatric cataracts

Elena V. Semina[1,2], Linda M. Reis[2], Maria R Replogle[2], Kailey Frank[2], Sarah Seese[2], Elena Sorokina[2]

[1]*Departments of Pediatrics and Cell Biology, Neurobiology and Anatomy, Medical College of Wisconsin, Milwaukee, WI, USA*
[2]*Department of Ophthalmology and Visual Sciences, Medical College of Wisconsin, Milwaukee, WI, USA*

Purpose: Accounting for about 10% of childhood blindness worldwide, pediatric cataracts are the chief cause of visual impairment in some nations, occurring at a rate of 2.2 to 13.6 per 10,000 births. Around 10–30% of childhood cataracts are believed to have a genetic origin. The diagnostic success rate for pediatric cataracts exhibits significant variation across different studies, with many papers reporting positive results in ~40–50% of families. This indicates the involvement of as-yet-unidentified mechanisms, the elucidation of which is crucial for improving diagnostic outcomes.

Methods: DNA samples from families affected with pediatric cataracts, with or without additional eye anomalies, and negative clinical genetic testing in many, underwent exome and/or genome sequencing. Variants were classified using American College of Medical Genetics and Genomics (ACMG) guidelines, incorporating updated in silico scoring guidelines. Potential splicing effects were investigated using the SpliceAI and Pangolin. Functional studies included animal models (zebrafish) and human cell culture using B3 lens epithelial cell line.

Results: Sequencing and variant data analyses identified a number of new alleles in known cataract genes. Several interesting/novel mechanisms have been identified including a new conversion involving *CRYBB2* and its pseudogene in one family; premature truncation variants in *DMRTA2* in two families; and splicing defects in *MPP5/PALS1* in two families. Zebrafish orthologs of dmrta2 and pals1 show strong expression during lens development. CRISPR-Cas9 technology was employed to create early truncation alleles within the *dmrta2* gene. While heterozygous *dmrta2* animals developed and survived normally, homozygous fish exhibited early lethality and brain defects. Functional analysis in cultured human lens cells confirmed the splicing abnormalities in *MPP5/PALS1* transcripts linked to the identified human variants.

Conclusion: We identified potential novel mechanisms of pediatric cataract, involving predicted loss-of-function variants in *DMRTA2* and *MPP5/PALS1* genes, as well as a new case of *CRYBB2* gene conversion.

Nanopore sequencing transcript analysis and functional investigation of a novel CYP51A1 genotype confirms the gene's association with autosomal recessive congenital cataract

Gavin Arno[1], Maxwell B Colonna[1], Andrzej B Poplawski[1], Marie N Brzoska[2], Dionne Le[2], Natasha L Rudy[1], Kameryn M Butler[1], Wesley G Patterson[1], Camerun C Washington[1], Elliot Stolerman[1], Libin Xu[2], Richard Steet[1]

[1]Greenwood Genetic Center, Greenwood, SC, USA
[2]Department of Medicinal Chemistry, University of Washington, Seattle, WA, USA

Purpose: There is emerging evidence implicating *CYP51A1*, encoding a lanosterol demethylase enzyme, in autosomal recessive pediatric cataract. Since 2012, biallelic variants in *CYP51A1* have since been identified in three unrelated families with congenital cataracts with or without variable liver disease, developmental delay and spastic diplegia. The association, however, remains uncertain. Here, we report genomic and functional investigations for a 21-month-old female with congenital cataracts with biallelic variants of uncertain significance in *CYP51A1*.

Methods: Clinical trio genome sequencing with rare variant prioritization was performed. Functional investigations were undertaken to resolve the impact of the *CYP51A1* variants, including Nanopore sequencing of transcript RT-PCR amplicons from primary cells of the child and both carrier parents, nonsense-mediated decay (NMD) blocking, immunoblotting to investigate protein abundance in cells and investigation of lanosterol accumulation and ferroptosis sensitivity.

Results: Two variants were identified in *CYP51A1*, c.316A>G, p.Met106Val (paternal) and c.468+1G>A (maternal). The paternally inherited variant caused a missense on the canonical isoform while being predicted to abolish translation of the alternate isoform due to start-loss. Transcript analysis showed a depletion of reads arising from the maternally inherited splicing variant. We identified a frameshift effect due to a 4-nucleotide intron inclusion on reads derived from this alelle. The resultant canonical isoform transcript was shown to undergo NMD while the alternate isoform was less depleted. The proband's cells showed a dramatically reduced enzyme abundance and a 5-fold lower enzymatic function resulting in lanosterol accumulation and increased sensitivity to ferroptosis.

Conclusions: We identified and functionally characterized a novel biallelic genotype in the *CYP51A1* gene in a child with congenital cataracts. Transcript analysis characterized the effect of the splice variant on each isoform and the contribution of NMD. Combined with the functional evidence, this provides significant evidence for the association of biallelic variants in the *CYP51A1* gene and congenital cataract.

Novel cataract risk loci identified from a large-scale multiethnic genome-wide association meta-analysis

Hélène Choquet[1,2], Chen Jiang[1], Vidhu Choudhary[1], Thomas J. Hoffmann[3,4], Poorab Sangani[5], and Salil A. Lachke[6,7], and Dima L. Chaar[1]

[1]Kaiser Permanente Northern California (KPNC), Division of Research, Pleasanton, CA, USA
[2]Department of Health Systems Science, Kaiser Permanente Bernard J. Tyson School of Medicine, Pasadena, California, USA
[3]Institute for Human Genetics, UCSF, San Francisco, CA, USA
[4]Department of Epidemiology and Biostatistics, UCSF, San Francisco, CA, USA
[5]KPNC, Department of Ophthalmology, South San Francisco, CA, USA
[6]Department of Biological Sciences, University of Delaware, Newark, DE, USA
[7]Center for Bioinformatics and Computational Biology, University of Delaware, Newark, DE, USA

Purpose: Cataract is the leading cause of blindness among the elderly worldwide. Twin and family studies highlight the important role of genetic factors to cataract risk, with heritability estimates up to 58%. To date, more than 100 cataract-associated loci have been identified by genome-wide association (GWA) studies, however much of cataract heritability remains unexplained. Here, we conducted a large-scale GWA meta-analysis of cataract (total sample size 1,244,025 individuals) to substantially improve genetic discovery.

Methods: We first conducted GWA analyses of cataract including 78,336 cataract cases and 222,108 controls from the large and ethnically diverse Kaiser Permanente Research Bank (KPRB) cohort. Logistic model GWA for cataract was conducted separately in each race/ethnicity group of the KPRB cohort (African American, East Asian, non-Hispanic white, and Hispanic/Latino), adjusting for age, sex, and ancestry principal components using REGENIE v3.6. Then, we performed a GWA meta-analysis combining our KPRB results with publicly available GWAS summary statistics for cataract, including 200,061 cataract cases and 1,043,964 controls. Finally, we conducted gene-based and pathways association analyses using Versatile Gene-based Association Study-2 version 2 (VEGAS2v02) integrative tool.

Results: Our multiethnic GWA meta-analysis identified 128 independent genome-wide significant ($P<5.0\times10^{-8}$) loci associated with cataract, of which 38 were novel. VEGAS2 gene-based association analysis prioritized 195 genes within the identified cataract-associated loci ($P<2.3\times10^{-6}$ = 0.05/21,402 genes tested). We also found 8 gene-set/pathways significantly enriched ($P<5.1\times10^{-6}$ = 0.05/9,734 pathways tested), including the Wnt signaling pathway.

Conclusions: The current study represents the largest and most ethnically diverse genetic study of cataract conducted to date to our knowledge. Study findings enhance our understanding of the genetic etiology of cataract risk by uncovering novel loci and biological pathways.

Aspartyl (Asparaginyl) beta-Hydroxylase deficiency impairs lens placode invagination and lens development in a Traboulsi syndrome Mouse Model

James Fielding Hejtmancik[1], Xiaodong Jiao[1], Lijin Dong[2], Robert Steven Lee[2], Brenda Klaunberg[3], Jeeva Munasinghe[3], Danielle Donahue[3], Kristofer Ervin[3], Collin Hodgkinson[4], David Goldman[4], Venkata Pulla Rao Vendra[1], S Amer Riazuddin[5], Zhiwei Ma[1]

[1]Ophthalmic Molecular Genetics Section, Ophthalmic Genetics and Visual Function Branch, National Eye Institute, Rockville, MD, USA
[2]NEI Genetic Engineering Core Facility, NEI, NIH, Bethesda, MD, USA
[3]Mouse Imaging Facility, National Institute of Neurological Disorders and Stroke, National Institutes of Health, 10 Center Drive B1D-69, Bethesda, MD, USA
[4]Laboratory of Neurogenetics, National Institute on Alcohol Abuse and Alcoholism, NIH, Bethesda, MD, USA
[5]The Wilmer Eye Institute, Johns Hopkins University School of Medicine, Baltimore, MD, USA

Purpose: Traboulsi syndrome is a rare autosomal recessive disease characterized by ectopia lentis, spontaneous filtering blebs, scleral thinning, anterior segment abnormalities, a distinctive facial appearance, and rarely cardiac and musculoskeletal involvement and microspherophakia. We studied the mechanism through which mutations in ASPH cause Traboulsi syndrome.

Methods: Whole genome and Sanger sequencing was used to identify mutations in human families. A knockout mouse was constructed using CRISPR/Cas9 technology and studied with multiple imaging approaches, confocal microscopy, whole transcriptome sequencing, and biochemical approaches.

Results: We identified an ASPH NM_004318.4: c. 2181_2183dupATG (CCDS34898.1) p. W728* mutation in a consanguineous Pakistani family with autosomal recessive ectopia lentis and modeled this with aspartyl (Asparaginyl) beta-Hydroxylase KO mice having a p.met653thrfsTer36 mutation eliminating the carboxyl-encoded aspartyl/asparaginyl β-hydroxylase domain. These mice exhibit significantly short body length, short snouts, deviated nasal septa, and a hunchback posture. Most KO mice exhibit unilateral or bilateral microphthalmia with aphakia. Those that develop lenses are emmetropic but recapitulate the Traboulsi human eye phenotype including lens dislocation, spontaneous filtering blebs, and iridocorneal adhesions. Additionally, they have cataracts. KO mice show disarray of collagen III fibers with decreased adhesion to the lens pit resulting in failure of lens invagination with lack of lens vesicle formation and subsequent microphthalmia in most. Mice with successful lens vesicle formation show emmetropic eyes with posteriorly dislocated lenses with incomplete primary fiber cell elongation resulting in lens opacities. Whole transcriptome sequencing shows minimal differences in WT and KO mouse lenses. Asph hydroxylates two sites on BmpI, increasing the rate at which it cleaves the C- terminal collagen propeptide from collagens I and III, the major collagens of the ECM connecting the lens pit and optic cup.

Conclusions: This provides a mechanism for ASPH requirements in lens and eye development and its role in Traboulsi syndrome.

Single cell RNA-Seq in a zebrafish model of crim1 loss of function – uncovering gene regulatory networks and mechanisms for lens development

Anne Slavotinek[1,2], Tien Le[1], Jared A. Tangeman[3], Josiah Immanuel Rajasingh[1], Sarah Y. Coomson[4], Salil A. Lachke[4,5]

[1]Division of Human Genetics, Cincinnati Children's Hospital Medical Center, Cincinnati, OH, USA
[2]Department of Pediatrics, University of Cincinnati College of Medicine, Cincinnati, OH, USA
[3]Solomon H. Snyder Department of Neuroscience, Johns Hopkins University School of Medicine, Baltimore, MD, USA
[4]Department of Biological Sciences, University of Delaware, Newark, DE, USA
[5]Center for Bioinformatics and Computational Biology, University of Delaware, Newark, DE, USA

Purpose: *CRIM1* encodes a type I transmembrane protein that is critical for regulating β1 integrin in the lens epithelium (LE) and promoting adhesion between LE and fiber cells. We generated a zebrafish model of reduced *crim1* function with smaller, malformed lenses and increased thickness of the retinal ganglion cell (RGC) layer to study the role of *crim1* in eye morphogenesis.

Methods: We performed scRNA-Seq in dissected eyes from the *crim1*$^{-/-}$ mutants and controls at 48 hpf to study the gene regulatory networks (GRNs) involved in lens and eye formation. We analyzed a 31-cluster model, focusing our attention on the cell types with differential expression of *crim1*. We used the Comprehensive Multi-omics Platform for Biological InterpretatiOn (CompBio) tool that uses contextual language processing of the biomedical literature to identify "enriched" biological themes among differentially expressed genes (DEGs).

Results: Our results showed dysregulation of integrin genes and genes involved in cell adhesion and apoptosis. l*gals2b*, orthologous to LGALS2 that was shown to bind β1 integrin in T cells, was the most upregulated gene in the Lens cluster. In the RGC cluster, *serpine3* that is associated with retinal layering and *sdk1b* that encodes a protein involved in cell-cell adhesion, were upregulated. *gpm6a* was downregulated in the RGC and RGC_2 clusters. CompBio identified themes associated with Neuronal differentiation and Actin cytoskeleton in the RGC cluster, Wnt signaling, Corneal structure and pathologies, and Sterol/cholesterol metabolism in the Corneal cluster, Stress activated signaling, Hypoxia response, Neuronal microtubules in the RGC_2 cluster, Axon pathfinding, Apoptosis, Cell cycle regulation, and ECM organization in the NC cluster.

Conclusions: Our results support the known function of *crim1* in integrin binding, cell adhesion and apoptosis, but also uncover novel, less studied DEGs and GRNs that are involved in these processes, including *lgals2b, serpine3, sdk1b*, and *gpm6a*, among others.

Tuesday, Dec. 9th

Tuesday, December 9th

Session 5: Lens Signaling

Moderators: *Xin Zhang & David Li*

Talks are in the format of 15 minutes with 3 minutes discussion.

Time	Speaker	Title
8:30 am	**David Li**	Regulation of Lens Development by Pax6 Sumoylation
8:48 am	**Haotian Lin**	Digital Twin of the Lens and AI-Assisted Diagnosis and Treatment of Cataracts
9:06 am	**Xiangjia Zhu**	Pathogenetic Mechanisms of Highly Myopic Cataract
9:24 am	**Qian Wang**	Dynamic regulation of FGF signaling in lens development
9:42 am	**Michael Karin**	Oxidized mitochondrial DNA and systemic autoimmune diseases

Regulation of Lens Development by Pax6 Sumoylation

David W Li[1], Shuyu Zheng[1], Yuyan Shan[1], and Jingmiao Wang[1]

[1]State Key Laboratory of Ophthalmology, Zhongshan Ophthalmic Center, Sun Yat-Sen University, # 7 Jinsui Road, Zhujiang New Town, Guangzhou, Guangdong, China

Purpose: Pax6 is a master regulator governing development of both brain and eye. Our previous studies have shown that SUMO1-mediated sumoylation is an indispensable step towards activation of p32 Pax-6 (Yan et al. 2010. PNAS). However, how does the sumoylated Pax6 regulate lens development is largely unknown.

Methods: CRISPR/Cas9 technology is used to create the mouse model where the conserved sumoylation site, Lys-91 was mutated into Arg-91 so that the conserved sumoylation site was abolished. RNAseq analysis was used to analyze the transcriptional activity of the mutated Pax6. QRT-PCR were used to confirm mRNA expression of Pax6 and other downstream genes in both wild type and mutant mice. Single cell sequencing was used to further explore the molecular mechanisms underlying abnormal development and cataractogenesis.

Results: K91R mutation abolishes in vivo sumoylation of Pax6 at K91 residue and causes abnormal lens development and cataractogenesis. Mechanistically, K91R mutation changes the stability of Pax6, leading to altered transcriptomes and changed cell subpopulations of the ocular lens.

Conclusions: Inhibition of in vivo sumoylation of Pax6 at K91 interrupts normal lens development, leading to cataractogenesis. Supported by National Natural Science Foundation of China (Grants #82271071; #81970787, #82000876, #81770910) and Natural Science Foundation of Guangdong Province and Guangdong City Joint Program of China (Grant # 2019B1515120014), and the Fundamental Funds, 3030901010110 of the State Key Laboratory of Ophthalmology of Zhongshan Ophthalmic Center.

Digital Twin of the Lens and AI-Assisted Diagnosis and Treatment of Cataracts

Haotian Lin[1,2,3], Xiaohang Wu[1], Lixue Liu[1], Xi Chen1, Naiji Y[1]

[1]Zhongshan Ophthalmic Center, Sun Yat-sen University, WHO Collaborating Centre for Eye Care and Vision, State Key Laboratory of Ophthalmology, Guangdong Provincial Key Laboratory of Ophthalmology and Visual Science, Guangdong Provincial Clinical Research Center for Ocular Diseases, Guangzhou, Guangdong, China.
[2]Hainan Eye Hospital and Key Laboratory of Ophthalmology, Zhongshan Ophthalmic Center, Sun Yat-sen University, Haikou, Hainan, China
[3]Centre for Precision Medicine and Department of Genetics and Biomedical Informatics, Zhongshan School of Medicine, Sun Yat-sen University, Guangzhou, Guangdong, China

Purpose: To delineate how DT technology redefines cataract therapeutics through multiscale integration—from molecular interactions to clinical outcomes—while establishing a framework for broader biomedical applications.

Methods: Digital modeling techniques could reconstruct the three-dimensional spatial typing system of the lens, showcasing the spatial phenotypic characteristics of the anterior, middle, and posterior cortices. Moreover, digital technology facilitates the construction of time-series lens age models, simulating the temporal phenotypic features of the lens at different developmental stages. It is worth noting that the digital mapping relationship between genetic anomalies and phenotypes is not static but dynamically evolves with the different stages of lens development. Therefore, in addition to digitizing the microstructural features of static tissue, it is also essential to digitally model dynamic characteristics, such as eye movements, to truly bridge the digital pathway from the micro to the macro and from static to dynamic aspects of the crystalline lens.

Results: DT technology embodies a fundamental reimagining of how we interrogate biological complexity. For cataracts, DT dissolves the artificial boundaries between molecular mechanisms and clinical phenotypes, offering a pathway to predictive, preventive, and personalized ocular therapeutics.

Conclusions: By enabling bidirectional mapping between biological systems and their digital counterparts, DT bridges the gap between the macroscopic and microscopic realms, offering unprecedented capabilities to simulate, analyze, and optimize cataract pathophysiology and treatment within a virtual environment.

Pathogenetic Mechanisms of Highly Myopic Cataract

Xiangjia Zhu[1,2,3,4], Dongling Guo[1,2,3,4], Yu Du[1,2,3,4], Ling Wei[1,2,3,4], Xin Liu[1,2,3,4], Dan Li[1,2,3,4]

[1]Eye Institute and Department of Ophthalmology, Eye & ENT Hospital, Fudan University, Shanghai, China
[2]Key Laboratory of Myopia and Related Eye Diseases, NHC Key Laboratory of Myopia and Related Eye Diseases, Chinese Academy of Medical Sciences, Shanghai, China
[3]Shanghai Key Laboratory of Visual Impairment and Restoration, Shanghai, China
[4]State Key Laboratory of Medical Neurobiology, Fudan University, Shanghai, China

Purpose: To investigate why highly myopic cataract (HMC) is characterized by a larger lens, earlier onset, and increased severity.

Methods: To elucidate the pathogenetic mechanisms of HMC from multiple dimensions, we compared lens size, crystallin protein expression, ferroptosis sensitivity, lens fiber polarity, and m6A methylation between HMC and age-related cataract (ARC) samples. Multiple experimental methods were employed, including high myopia mouse model construction, mRNA sequencing, m6A-modified RNA immunoprecipitation, primary cell culture, siRNA interference, western blotting, co-immunoprecipitation, immunofluorescence.

Results: We found that highly myopic eyes had enlarged lenses, as revealed by magnetic resonance imaging. This enlargement was associated with upregulated expression of β/γ-crystallin, driven by overexpression of the transcription factor musculoaponeurotic fibrosarcoma oncogene (MAF). We further discovered that increased sensitivity to ferroptosis contributes to the earlier onset of cataracts in highly myopic lenses. In lens epithelia of HMC, Fe^{2+} levels and lipid peroxidation were elevated, and both transmission electron microscopy and lens explant incubation confirmed enhanced vulnerability to ferroptosis. Overexpression of discoidin domain receptor tyrosine kinase 2 (DDR2) emerged as a key driver of this process by promoting ferroptosis sensitivity through the Src–Hippo pathway. In addition, we identified that the nuclear phenotype of highly myopic lenses results from enhanced polarity of lens fibers. Elevated transforming growth factor-β1 (TGF-β1) in HMC induced increased m6A modification, which further promoted fiber migration and polarity by regulating the planar cell polarity (PCP) pathway.

Conclusions: Our findings reveal that unique regulatory mechanisms in highly myopic lenses, including crystallin overexpression, heightened ferroptosis sensitivity, and enhanced fiber polarity, jointly drive the characteristic features of HMC: larger lens, earlier onset, and increased severity of opacification.

Dynamic regulation of FGF signaling in lens development

Qian Wang[1]

[1]*John F. Hardesty, MD Departments of Ophthalmology, Washington University in St. Louis, Saint Louis, MO, USA*

Purpose: Fibroblast Growth Factor (FGF) signaling is critical for multiple stages of lens development. This signal is not constitutively active but displays dynamic oscillations between active and inactive states. However, the mechanisms underlying these dynamics remain poorly understood.

Methods: We use lens-specific conditional knockout and CRISPR-generated knock-in mouse models. Embryos are harvested and analyzed by immunostaining.

Results: We observed distinct and dynamic spatiotemporal patterns for FGFR1 and FGFR2 in the developing eye, which are not fully explained by their mRNA patterns. Specifically, FGFR1 was absent in the surface ectoderm but was induced in the invaginating lens pit, where FGF signaling is highly active. While FGFR2 was expressed in the surface ectoderm but was dramatically downregulated in the lens pit. By E13.5, FGFR1 localized primarily to the lens transition zone, while FGFR2 mainly in the lens epithelium.

We further investigate the potential mechanism for FGFR2 dynamics by targeting a conserved endocytic motif, YXXL present in all FGFRs (Y, tyrosine; X, any animo acid; L, leucin). Mouse carrying FGFR2 tyrosine to phenylalanine mutation (*Fgfr2*^{Y769F}) developed persistent lens-corneal attachment, a phenotype mimicking type II Peters Anomaly in humans. Existing models suggest that Peters Anomaly is associated with FGF gain-of-function rather than loss-of-function. Consistent with this, we found elevated FGFR2 expression and enhanced FGF signaling in the developing lens and in cultured lens epithelial cells derived from *Fgfr2*^{Y769F} mutants. This gain-of function phenotype positions *Fgfr2*^{Y769F} as a valuable model to study the mechanisms of FGFR2 endocytosis and its *in vivo* significance.

Conclusions: The spatiotemporal divergence of FGFR1 and FGFR2 suggests that these receptors are differentially regulated by FGF signalling, representing a potential feedback mechanism for precise control of FGF signaling output. The steady-state level of FGFR2 is controlled by FGF-induced endocytosis, a process dependent on Y769. The regulatory mechanism for FGFR1 remains to be determined.

Oxidized mitochondrial DNA and systemic autoimmune diseases

Michael Karin[1]

[1]*SBP Discovery, La Jolla CA, USA*

Oxidized mitochondrial DNA (Ox-mtDNA) is an alarmin produced in response to mitochondrial stress whose circulating amounts are elevated in the elderly and in patients with different systemic autoimmune and metabolic diseases. In the past we have shown that Ox-mtDNA binds to NLRP3 and leads to activation of the corresponding inflammasome, resulting in IL-1b and IL-18 secretion from macrophages stimulated with several different NLRP3 inflammasome activators. After working out the pathway responsible for translocation of Ox-mtDNA fragments from the mitochondrion to the cytosol we found that a portion of these fragments are released to the extracellular milieu and enter the circulatory system. Of note, circulating Ox-mtDNA leads to activation of plasmacytoid dendritic cells (pDC) while having little effect on conventional DC. pDC that have been activated by Ox-mtDNA, but not by non-oxidized mtDNA acquire the ability to convert naïve CD4+ T cells to follicular helper T cells (Tfh), which then stimulate the production of autoantibodies by germinal center B cells. This cascade of events results in the development of a lupus-like autoimmune disease in mice. Accordingly, we propose that circulating Ox-mtDNA in humans underlies the pathogenesis of systemic autoimmune diseases, as well as age-related neurogenerative and autoinflammatory diseases. References: Zhong et al., Nature 2018; Xian et al., Immunity 2022: Xian et al., Nature Immunology 2025.

Tuesday, December 9th

Session 6: Crystallin Modifications & Cataractogenesis

Moderators: *Kirsten Lampi & Eugene Serebryany*

Talks are in the format of 15 minutes presentation with 3 minutes discussion.

Time	Speaker	Title
10:30 am	**Eugene Serebryany**	Investigating the mechanism of suppression of γ-crystallin misfolding and aggregation by the abundant lens metabolite myo-inositol
10:48 am	**Adam Miller**	α-crystallins play the long game
11:06 am	**Laxman Mainali**	Aggregation of α-Crystallin to Lens Membrane Increases with Peroxidation of Lipid and Cholesterol
11:24 am	**Constance Kraay**	Unfolding Intermediates of γD-Crystallin and γS-Crystallin Identified *in silico* Provide Insights into Mechanisms and Suppression of Aggregation
11:42 am	**Kirsten Lampi**	Stepwise Mechanism of Crystallin Insolubilization due to the Synergestic Effects of Deamidation and Oxidation

Investigating the mechanism of suppression of γ-crystallin misfolding and aggregation by the abundant lens metabolite myo-inositol

Eugene Serebryany[1,2,3,4], Loy Hashimoto[1,2], Yu Pu[1,2,3]

Stony Brook University, SUNY, Stony Brook, NY, USA
[1]Department of Physiology & Biophysics
[2]Laufer Center for Physical & Quantitative Biology
[3]Department of Chemistry
[4]Stony Brook Cancer Center

Purpose: Assembly of post-translationally damaged γ-crystallins into large light-scattering aggregates in the aging lens is a major contributing factor to cataract disease, one of the most prevalent protein aggregation disorders. Therapeutic options for human age-onset cataract are still lacking, partly because delivering aggregation suppressors (or any drugs) to the lens is notoriously difficult. However, the lens natively imports and concentrates the small cyclic sugar alcohol myo-inositol, which has been shown to suppress cataract-associated γ-crystallin aggregation in vitro. The mechanism of this aggregation suppression is a topic of active investigation in our lab.

Methods: We quantified the ability of myo-inositol and several structurally or chemically similar compounds to suppress light scattering aggregation of misfolding-prone human γD-crystallin variants. We generated monomers and disulfide-bridged dimers of the isolated N-terminal domain of human γD-crystallin, which exhibits redox- and temperature-dependent aggregation. We measured aggregation suppression by myo-inositol as a function of the extent of dimerization to determine how early in the aggregation process the chemical chaperone acts.

Results: Myo-inositol strongly suppressed aggregation of the γD-crystallin N-terminal domain. Prior investigations revealed that myo-inositol's primary target was not the native, unfolded, or final aggregated states of the protein. In the present experiments, monomers and dimers aggregated to a similar extent, but the degree of dimerization anti-correlated with the degree of aggregation suppression by myo-inositol.

Conclusions: Myo-inositol acts at the earliest stage of the aggregation pathway, predominantly on the misfolding monomer. Misfolding of either subunit within a dimer likely enables aggregation. Thus, a dimer has two chances to escape myo-inositol suppression, while the monomer has one.

α-crystallins play the long game

Adam Miller[1]

[1]*Oregon Health Sciences University*

The α-crystallins (αAc and αBc) utilize a chaperone function in the lens to prevent protein aggregation and cataract formation by forming soluble chaperone/client complexes. Over time, the accumulation of chaperone/client complexes depletes the pool of available chaperones and may disrupt the balance of crystallin proteins packed in the lens. The structurally dynamic and heterogeneous assemblies of α-crystallins – and other small heat shock proteins (sHSPs) – have stymied attempts to describe their mechanism of chaperone function. Structural heterogeneity becomes even more apparent when forming complexes with clientele. Negative-stain EM, Cryo-EM, and other biophysical approaches propose multiple mechanisms of chaperone function used by the α-crystallins and the archaeal HSP16.5 homolog. Quantitative morphological measurements (e.g., Feret diameter and Area) of chaperone/client complexes directly from negative-stain EM micrographs show a quasi-ordered expansion and elongation mechanism of α-crystallin "holdase" function to prevent the aggregation of the model client's lysozyme and insulin. This mechanism was conserved for heterologously expressed αAc and αBc homo-oligomers and the hetero-oligomeric αLc (αAc/αBc) isolated from sheep lenses. Single-particle Cryo-EM reconstructions and structure-guided mutational studies of HSP16.5 propose a mechanism of oligomeric plasticity enabling elongation involving phenylalanine residues in the N-terminal domain that are conserved in α-crystallins. Furthermore, Cryo-EM reconstructions of an ensemble of HSP16.5 client-bound oligomeric states showcase a mechanism of cage elongation through stability-polarization of the sHSP scaffold. This multi-modal structural approach has provided insights into both the elusive N-terminal domain supporting oligomeric plasticity and the morphological transitions taken by sHSPs to accommodate clientele and prevent aggregation.

Aggregation of α-Crystallin to Lens Membrane Increases with Peroxidation of Lipid and Cholesterol

Laxman Mainali[1,2], Nawal K. Khadka[1], Preston Hazen[2], Oluwaseyi Akinola[2], and Xinzhu Pu[3]

[1]Department of Physics, Boise State University, Boise, ID, USA
[2]Biomolecular Sciences Graduate Program, Boise State University, Boise, ID, USA
[3]Biomolecular Research Center, Boise State University, Boise, ID, USA

Purpose: α-Crystallin (αABc), comprising αA-crystallin (αAc) and αB-crystallin (αBc) subunits, is found to form membrane-bound aggregates with aging and cataract formation. However, the molecular basis for such aggregate formation is still unclear. This study investigates the role of lipid and cholesterol (Chol) peroxidation in the aggregation of αAc, αBc, and αABc with lens cortical membrane (CM) and nuclear membrane (NM).

Methods: The topographical images of cataractous human lens CM and NM with and without αAc and bovine lens NM and oxidized-NM (Ox-NM) with and without αAc, αBc, or αABc were obtained using atomic force microscopy (AFM) and correlated with lipid plus Chol peroxidation. The percent membrane surface occupied (%MSO) by αAc and the mobility and order near the headgroup regions with αAc binding to NM and Ox-NM were measured using electron paramagnetic resonance (EPR).

Results: With lipid and Chol peroxidation in bovine lens NM, the aggregation of αAc, αBc, or αABc on Ox-NM was significantly more extensive, and aggregation increased with an increase in lipid and Chol peroxidation. The average area of αAc aggregates formed on the cataractous human lens NMs was found to be significantly higher than that in the cataractous human lens CM, and correlated with higher lipid plus Chol peroxidation in NMs compared to CM. The %MSO by αAc on Ox-NM was found to be significantly higher than that of unoxidized NMs. The oxidation increased mobility and decreased membrane order near the headgroup regions, while the binding of αAc to Ox-NM significantly decreased mobility near the headgroup regions.

Conclusions: The aggregation of αAc, αBc, and αABc on lens CM and NM increases with increased lipid and Chol peroxidation, and the formation of such large aggregates likely promotes increased light scattering and cataract formation.

Unfolding Intermediates of γD-Crystallin and γS-Crystallin Identified *in silico* Provide Insights into Mechanisms and Suppression of Aggregation

Constance Kraay[1], Srivastav Ranganathan[2], Kate Halverson-Kolkind[3], Larry David[3], Kirsten Lampi[3], Eugene Serebryany[4], Eugene Shakhnovich[5]

[1]Harvard Graduate Program in Biophysics, Harvard University, Cambridge, MA, USA
[2]Max Planck Institute for the Physics of Complex Systems, Dresden, Germany
[3]Department of Integrative Biosciences, Oregon Health & Science University, Portland, OR, USA
[4]Department of Physiology and Biophysics and the Laufer Center for Physical and Quantitative Biology, Stony Brook University, Stony Brook, NY, USA
[5]Department of Chemistry and Chemical Biology, Harvard University, Cambridge, MA, USA

Purpose: The aggregation of the γD-crystallin and γS-crystallin proteins in the lens is implicated in cataract disease. Aggregation is thought to occur from misfolded states, and computational approaches can complement experiments by providing atomistic insights into these non-native states. In this work, computational techniques were used to investigate two disease-relevant perturbations to human γ-crystallins: (1) myo-inositol, a metabolite that is abundant in healthy lenses but not in cataractous ones, suppresses aggregation of γD-crystallin in vitro, and (2) deamidations alter the conformational dynamics and aggregation propensity of γS-crystallin.

Methods: Monte Carlo (MC) simulations were used to generate unfolding intermediates of the N-terminal domain of γD-crystallin, and interactions between myo-inositol and these states were identified using Molecular Dynamics simulations. In addition, theoretical hydrogen/deuterium exchange mass spectrometry (HDX-MS) spectra were calculated from MC simulations of γS-crystallin and compared with experimental data, allowing populations of partially unfolded states to be identified. Unfolding intermediates from MC simulations were also compared with structures obtained by perturbing multiple sequence alignment inputs to AlphaFold.

Results: Unfolding pathways for γD-crystallin were identified, and myo-inositol was found to preferentially bind to a partially unfolded state over the native state, in line with previous experimental results. A potential binding site on this intermediate suggested a mechanism for suppression of aggregation. In addition, HDX-MS spectra calculated from simulations revealed that the triply-deamidated form of γS-crystallin had a more polydisperse unfolded ensemble than the wild-type protein, potentially leading to higher aggregation propensity.

Conclusions: A mechanism through which myo-inositol suppresses γD-crystallin aggregation by binding to a non-native state was proposed, and deamidations were found to perturb partially unfolded states of γS-crystallin. Taken together, these results demonstrate how computational approaches can complement experiments by providing atomistic insights into processes in which partially unfolded states play a significant role, such as crystallin aggregation in the lens.

Stepwise mechanism of oxidation of gammaS-crystallin leading to aggregation

Kirsten J. Lampi[1], Larry L. David[1], Kate Halverson-Kolkind[1], Martin Tovar Ramirez[1], Constance Kraay[2], Eugene Shakhnovich[3], David Thorn[3]

[1]Oregon Health & Science University, Portland, OR, USA
[2]Harvard University Graduate Program in Biophysics, Boston, MA, USA
[3]Harvard Univ Dept Chemistry, Cambridge, MA, USA

Purpose: Crystallins accumulate numerous post-translational modifications during aging and are resistant to denaturation. During aging, the pool of lens glutathione diminishes in the center of the lens making sulfhydryls in crystallins more susceptible to oxidation. The role of oxidized glutathione in crystallin aggregation was probed in this study.

Methods: Crystallins were subjected to oxidation in the presence of oxidized glutathione and then heated to further mimic aging. Cysteine knock-out mutants were analyzed to confirm the role of specific cysteines. High resolution mass spectrometry was then used to identify the crosslinks.

Results: We identified a stepwise mechanism of disulfide crosslinking in γS-crystallin involving oxidation at the exposed cysteine at residue 24 followed by a stabilizing crosslink between cysteines 22 and 26. Upon heating, this major species was diminished while other non-native crosslinks involving cysteines at residues 36 and 82 increased.

Conclusions: These data provide a strong premise for our hypothesis that while the Cys22-Cys26 disulfide bond is protective against insolubilization, further oxidation facilitates non-native disulfide crosslinking initiating aggregation. Such findings are crucial for establishing causality between the cumulation of post-translational modifications and cataract. Therapeutic intervention early with antioxidants targeted at specific sites may slow the onset of cataract.

Funding: NEI EY027012

Tuesday, December 9th

Session 7: Lens Clinical Studies

Moderators: *Hiroshi Sasaki & Eri Kubo*

Talks are in the format of 15 minutes presentation with 3 minutes discussion.

Time	Speaker	Title
1:15 pm	**Hiroyuki Matsushima**	Biocompatibility and Functionality of Intraocular Lens Materials
1:33 pm	**Yoichiro Masuda**	From Atopic Cataract to IOL Dislocation: A Pathological Journey Through the Lens Capsule
1:51 pm	**Andrew Smith**	LEFTY2 is an Aqueous Humor Biomarker for Exfoliation Glaucoma
2:09 pm	**Takayoshi Sumioka**	Dead Bag Syndrome after Cataract Surgery: Pathological Findings and a Representative Case
2:27 pm	**Takashi Ono**	Optimizing Systemic Care in Cataract Surgery for the Super-Aged Society

Biocompatibility and Functionality of Intraocular Lens Materials

Hiroyuki Matsushima, MD, PhD[1]

[1]*Department of Ophthalmology, Dokkyo Medical University, Tochigi, Japan*

Purpose: The main materials used for intraocular lenses (IOLs) are hydrophobic and hydrophilic acrylics. Hydrophobic acrylics are favored for their compatibility with small-incision surgeries and long-term stability. In contrast, hydrophilic acrylics offer high biocompatibility but are prone to calcium deposition. This study aims to analyze the specific issues associated with each material type.

Methods: Hydrophobic acrylics are widely used and are considered the mainstream material for IOLs globally. While their long-term stability is well recognized, they are susceptible to phase separation in aqueous environments, leading to phenomena such as glistening and sub-surface nano glistening. Although recent improvements in manufacturing processes have reduced lens opacification, a significant number of IOLs manufactured by older methods are still in use and show notable clouding.

Results: Hydrophilic acrylics, being soft and highly biocompatible, have also attracted attention. However, calcium deposition remains a critical concern, especially after procedures like corneal transplantation or vitrectomy. Studies in other fields suggest that carboxyl (COOH) groups are involved in calcium deposition, and surface methylation of hydrophilic materials may suppress this process.

Conclusions: While IOL materials have evolved to become safer and more stable over the long term, several unresolved issues remain. Continued basic and clinical research is essential for the development of improved IOL materials in the future.

From Atopic Cataract to IOL Dislocation: A Pathological Journey Through the Lens Capsule

Yoichiro Masuda[1]

Atopic cataract differs markedly from age-related cataract in both onset and pathology. It typically develops at a younger age, with a thinner lens and, in some cases, dense anterior capsular calcification. Rapid progression to white, intumescent cataract is not uncommon, and postoperative complications such as intraocular lens (IOL) dislocation are more frequent. In this presentation, I will highlight the unique clinicopathological characteristics of atopic cataract, focusing on pathological findings of the lens capsule and epithelial cells, and discuss how these insights may explain the link between atopic cataract and late in-the-bag IOL dislocation.

LEFTY2 is an Aqueous Humor Biomarker for Exfoliation Glaucoma

Andrew J. O. Smith[1], Steven Bassnett[1]

[1]*Department of Ophthalmology and Visual Sciences, Washington University School of Medicine, St. Louis, MO, USA*

Purpose: Exfoliation syndrome (XFS) is characterized by the production of fibrillar exfoliation material (XFM) by the lens and other tissues of the anterior segment. XFM accumulates on the lens, iris, ciliary body, and notably the trabecular meshwork, where it is believed to impede aqueous humor outflow. Patients with XFS often develop cataracts and exfoliation glaucoma (XFG), a severe form of glaucoma for which no reliable biomarkers exist. Here, we evaluated Left-right determination factor 2 (LEFTY2), a TGFβ family member with no recognized ocular role, as a potential biomarker for XFG.

Methods: Aqueous humor was collected from patients undergoing cataract surgery with informed consent. The exfoliation groups included XFS (n = 7) and XFG (n = 22). The reference groups included cataract only (CAT, n = 78) and cataract with POAG (CAT/GL, n = 67). LEFTY2 concentration was measured by ELISA, and XFM burden was graded clinically by slit-lamp examination. Human trabecular meshwork (HTM) cells were generated and treated with TGFβ isoforms; LEFTY2 expression was determined by RT-qPCR and ELISA.

Results: LEFTY2 was significantly elevated in the aqueous humor of XFG patients (XFG: 27.23 ± 45.39 ng/ml; XFS: 4.00 ± 4.07 ng/ml; CAT: 1.19 ± 0.72 ng/ml; CAT/GL: 1.68 ± 1.81 ng/ml). ROC analysis showed that LEFTY2 levels discriminated XFG from reference groups with high accuracy (CAT vs. XFG; AUC = 0.87, $p < 0.0001$). Aqueous humor LEFTY2 concentration correlated with the extent of XFM deposition. In vitro, HTM cells treated with TGFβ showed significantly increased LEFTY2 expression.

Conclusions: XFG is an aggressive form of glaucoma that currently lacks validated biomarkers. Our findings indicate that LEFTY2 is strongly associated with exfoliation glaucoma and may serve as a promising aqueous humor biomarker for this potentially sight-threatening condition.

Dead Bag Syndrome after Cataract Surgery: Pathological Findings and a Representative Case

Takayoshi Sumioka[1], Liliana Werner[2], Shingo Yasuda[1], Hiroki Iwanishi[1], Shizuya Saika[1]

[1]Department of Ophthalmology, Wakayama Medical University School of Medicine, Wakayama-shi, Japan
[2]Department of Ophthalmology and Visual Sciences, John A. Moran Eye Center, University of Utah, Salt Lake City, UT, USA

Cataract surgery is an extremely effective surgical procedure for restoring visual function; however, the most frequent postoperative complication is posterior capsule opacification (PCO). PCO arises from the fibrosis of residual lens epithelial cells (LECs) and is well recognized as a condition that can compromise the stability of intraocular lenses (IOLs). In contrast, the recently reported entity known as Dead Bag Syndrome (DBS) is characterized by a marked reduction or loss of LECs, resulting in an excessively transparent and floppy capsular bag that may lead to IOL decentration or dislocation. Immunohistochemical analyses of DBS specimens have demonstrated poor expression of fibrous components such as type I collagen, fibronectin, and vimentin, providing histological evidence of LEC depletion. Potential etiologies include early disappearance of LECs, effects of aqueous humor components, delamination of the capsular bag, and atopic predisposition, although the definitive mechanism remains unclear. In this presentation, we report the distinctive pathological features of DBS and illustrate its clinical significance through a representative case.

Optimizing Systemic Care in Cataract Surgery for the Super-Aged Society

Takashi Ono[1]

[1]*Department of Ophthalmology, University of Tokyo, Tokyo, Japan*

Purpose: As populations age worldwide, cataract surgery is increasingly performed in patients with advanced age and multiple comorbidities. Compared with younger individuals, elderly patients often present with ophthalmic risk factors such as zonular laxity, pseudoexfoliation, and poor pupillary dilation, while also carrying a higher burden of systemic disease. Uncontrolled hypertension during the perioperative period can lead not only to increased intraoperative bleeding but also to life-threatening cardiovascular and cerebrovascular complications.

Methods: We developed a collaborative care model integrating ophthalmologists, internists, and nursing staff to address systemic risk in elderly cataract patients. Structured protocols emphasized early and active management of blood pressure, with additional monitoring of heart rate to maintain hemodynamic stability. The impact of metabolic disorders such as hypertension and dyslipidemia was also evaluated. Surgical outcomes in centenarians were examined to assess feasibility in extreme age.

Results: Standardized perioperative management effectively blunted rises in systolic and diastolic pressure and reduced pulse pressure variability. Incorporation of heart rate monitoring further decreased episodes of hypertensive crisis. Metabolic syndrome did not appear to worsen perioperative hemodynamics when systemic control was optimized. Importantly, cataract surgery was performed safely in patients over 100 years of age, demonstrating that chronological age alone should not preclude surgery when systemic risks are properly managed.

Conclusions: Perioperative systemic management is central to safe cataract surgery in the super-aged population. Coordinated control of blood pressure and heart rate provides tangible protection against intraoperative and systemic complications. With appropriate protocols in place, cataract surgery can be undertaken safely even in the oldest old. Ongoing work is directed toward refining these strategies to meet the growing demand for surgical care in an aging society.

Tuesday, December 9th

Time	Title
3:00 pm	Early Career Cataract Research Award Presentation
3:05 pm	NFER Cataract Award Talk (Dr. Xiangjia Zhu)
3:30 pm	Poster Lightning Talks (Nick Delamere)
4:15 pm	Poster Session

Posters

Poster No.	Speaker	Title
1.	**Venkata Pulla Rao Vendra**	The cataract associated mutation G165R of human beta B3-crystallin destabilizes the molecule and promotes aggregation
2.	**Keke Zhang**	Comparative Phosphoproteomic Profiling of Lenses from Patients with Highly Myopic Cataract and Age-Related Cataract
3.	**Hongzhe Li**	Proteomic characterization of capsular fibrosis and the role of NPC1 in TGF-β2-induced epithelial-mesenchymal transition in lens epithelial cells
4.	**Atsushi Tohma**	Immunohistochemical Characteristics of Advanced Glycation End-products (AGEs) in the Lens Capsule in Intraocular Lens Dislocation
5.	**Rylee King**	The Role of Actin Binding protein, TAGLN, in Myofibroblast Differentiation of Lens Epithelial Cells
6.	**Anna Lubertozzi**	Afadin Orchestrates Tricellular Adherens Junctions and Lens Tissue Architecture
7.	**Electra Coffman**	Arvcf stabilizes adherens junctions and Ankyrin-B protein within the lens fiber cell membrane
8.	**Yuka Koshiishi**	Unilateral Pediatric Cataract with a Thinned Crystalline Lens: A Case Report

Poster No.	Speaker	Title
9.	**Mayumi Nagata**	A Case of Lamellar Separation of the Lens Capsule During Cataract Surgery
10.	**Natsuko Hatsusaka**	Cataract Survey in an Epidemiological Study on the Health Effects of Occupational Radiation
11.	**Koichiro Mukai**	Exploration of Hydrophilic Materials Less Prone to Calcium Deposition
12.	**Kohei Miyata**	Pupillary responses in eyes implanted with multifocal and monofocal intraocular lenses
13.	**Norihiro Watanabe**	Dynamic Pupillary Responses in Multifocal Intraocular lens (Synergy® and Odyssey®) Implanted Eyes
14.	**Natsuko Maeda**	Dynamic Pupillary Response and Accommodation with Intraocular Lenses and Crystalline Lenses
15.	**Reona Mouri**	Correlation with Lens accommodation parameters and Retinal age
16.	**Yurui Pan**	Cluster Analysis Identifying High-Risk Biometric Combinations for IOL Calculation Error in High Myopia
17.	**Soyoka Goto**	Postoperative Outcomes of Cataract Surgery with Intraoperative Mydriasis in Eyes with Primary Angles-closure
18.	**Shan Yuyan**	Pax6-K91R Mutant Mice Display Changed Transcriptome and Disrupted Lens Differentiation as Revealed by Single Cell RNA Sequencing (scRNA-seq) Study
19.	**Pooja Rathaur**	LOXL1 Loss Strengthens Ciliary Zonules and Alters Lens Epithelium Transcriptome
20.	**Yu Du**	ANGPTL7 Elevation Drives Intraocular Pressure Dysregulation in Highly Myopic Cataractous Eyes: A Multi-omics Insight
21.	**Hirotaka Hashimoto**	Effects of astaxanthin intake on adiponectin level in human aqueous humor
22.	**Xue-Bin Hu**	The Protein Phosphatase 1 Regulator, MYPT1 Control Lens Development through Direct Modulation of Functions of HMGA1 and Other Downstream Targets

Poster No.	Speaker	Title
23.	**Jing-Miao Wang**	Heat Shock Protein 90β Modulates Lens Inflammatory Response to Prevent Cataractogenesis via Direct Interaction with NF-κB p65
24.	**Yuka Okada**	Effects of Tenascin-X Deficiency on Anterior Segment Morphology
25.	**Yosuke Nakazawa**	Lens sclerosis is promoted by Piezo1 channel through transglutaminase 2 activation

Poster 1

The cataract associated mutation G165R of human bB3-crystallin destabilizes the molecule and promotes aggregation

Venkata Pulla Rao Vendra[1], Mariia Chornobhai[1], Marzena Dyba[2], Sergey G. Tarasov[2], J. Fielding Hejtmancik[1]

[1]Ophthalmic Molecular Genetics Section, Ophthalmic Genetics and Visual Function Branch, National Eye Institute, National Institutes of Health, Bethesda, MD, USA
[2]Structural Biophysics Laboratory, National Cancer Institute, Frederick, MD, USA

Purpose: Lens transparency and focusing require lens crystallins to have high solubility and stability throughout the life of the organism. As mutations that destabilize crystallins are known to cause cataracts, we investigated the molecular mechanisms underlying autosomal dominant congenital nuclear cataracts associated with a G165R change in human bB3-crystallin.

Methods: Wild-type and mutant proteins were overexpressed in BL-21 DE3 bacterial cells, purified using standard chromatography techniques and their structure, stability and aggregation properties were characterized by fluorescence, circular dichroism and DLS methods.

Results: The G165R mutation alters the secondary and tertiary structural fold, slightly exposing the hydrophobic core. Under thermal stress the wild-type and mutant proteins showed a two-state transition. The mutant shows compromised thermal stability, with lower T_m. Both the wild-type and mutant show two-state transitions with GuHCl induced unfolding-refolding experiments. The mutant shows a decreased C_m of 0.51M and ΔG^0 of 0.513 Kcal.mol^{-1} whereas the wild-type shows 0.87M and 2.267513 Kcal.mol^{-1}, respectively. Only the mutant displays light scattering particles at 45°C between 200-300sec. Finally, the wild type exists as a monomer at concentrations below 0.2 mg/mL, but forms dimers above this concentration, while the mutant fails to form dimers.

Conclusions: The G165R mutation microenvironment in human-bB3 crystallin alters its backbone conformation, exposing the hydrophobic core to the surface and increasing its susceptibility to thermal and chemical induced denaturation, and resulting in lack of appropriate dimerization as well as self-aggregation and lens opacification.

Poster 2

Comparative Phosphoproteomic Profiling of Lenses from Patients with Highly Myopic Cataract and Age-Related Cataract

Keke Zhang[1,2,3], Shaohua Zhang[1,2,3], Wenwen He[1,2,3], Yi Lu[1,2,3], Xiangjia Zhu[1,2,3,4]

[1]*Eye Institute and Department of Ophthalmology, Eye & ENT Hospital, Fudan University, Shanghai, China*
[2]*NHC Key Laboratory of Myopia and Related Eye Diseases, Key Laboratory of Myopia and Related Eye Diseases, Chinese Academy of Medical Sciences, Shanghai, China*
[3]*Shanghai Key Laboratory of Visual Impairment and Restoration, Shanghai, China*
[4]*State Key Laboratory of Medical Neurobiology, Fudan University, Shanghai, China*

Purpose: To investigate and compare the lens phosphoproteomes in patients with highly myopic cataract (HMC) or age-related cataract (ARC).

Methods: In this study, we undertook a comparative phosphoproteomic profiling of the lenses from patients with HMC or ARC. Intact lenses from ARC and HMC patients were separated into the cortex and nucleus. After protein digestion, the phosphopeptides were quantitatively analyzed with TiO2 enrichment and liquid chromatography-mass spectrometry. The potential functions of different phosphopeptides were assessed by Gene Ontology (GO) enrichment analysis and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis.

Results: In total, 522 phosphorylation sites in 164 phosphoproteins were identified. The number of phosphorylation sites was significantly higher in the cortex than in the nucleus, in both ARC and HMC lenses. The differentially phosphorylated peptides in the lens cortex and nucleus in HMC eyes were significantly involved in the glutathione metabolism pathway. The KEGG pathway enrichment analysis indicated that the differences in phosphosignaling mediators between the ARC and HMC lenses were associated with glycolysis and the level of phosphorylated phosphoglycerate kinase 1 was lower in HMC lenses than in ARC lenses.

Conclusions: We provide an overview of the differential phosphoproteomes of HMC and ARC lenses that can be used to clarify the molecular mechanisms underlying their different phenotypes.

Proteomic characterization of capsular fibrosis and the role of NPC1 in TGF-β2-induced epithelial-mesenchymal transition in lens epithelial cells

Hongzhe Li[1,2], Han Zou[1,2], Xiaohui Jiang[1,2], Siyan Li[1,2,3], Yinying Zhao[1,2,3], Pingjun Chang[1,2,3], Yun-e Zhao[1,2]

[1]National Clinical Research Center for Ocular Diseases, Eye Hospital, Wenzhou Medical University, Wenzhou, China
[2]State Key Laboratory of Eye Health, Eye Hospital, Wenzhou Medical University, Wenzhou, China

Purpose: Fibrotic changes resulting from epithelial-mesenchymal transition (EMT) of lens epithelial cells (LECs) are common sequelae of cataract surgery and may be induced by various triggers. Fibrosis can also develop in the lens capsule (LC) of patients with age-related cataracts (ARC) without these established risk factors. This study aimed to identify key proteins involved in capsular fibrosis through proteomic profiling and to elucidate their functional roles in EMT progression in LECs.

Methods: Comparative proteomic analysis was performed on LC and aqueous humor samples from ARC patients with (n = 10) and without (n = 10) capsular fibrosis to identify target proteins. Human LECs were treated with TGF-β2 to induce EMT, and the expression of Niemann–Pick type C1 (NPC1) in fibrotic LC tissues and TGF-β2-treated human LECs was validated by Western blot. Additionally, human LECs were transfected with a plasmid encoding the NPC1 coding sequence, and the expression levels of EMT- and autophagy-related molecules were further analyzed.

Results: Proteomic analysis revealed significant downregulation of NPC1 and upregulation of TGF-β2 in both samples. Functional studies in human LECs showed that NPC1 overexpression attenuated TGF-β2-induced EMT, as evidenced by increased expression of epithelial markers (zonula occludens-1 and E-cadherin) and decreased expression of mesenchymal markers (N-cadherin and α-smooth muscle actin). Additionally, NPC1 overexpression enhanced autophagy, as indicated by reduced p62 expression and altered LC3-I/II ratios.

Conclusions: NPC1 was downregulated in fibrotic LC tissues and was found to modulate TGF-β2-induced EMT and autophagy in human LECs, highlighting potential targets for its prevention and treatment.

Poster 4

Immunohistochemical Characteristics of Advanced Glycation End-products (AGEs) in the Lens Capsule in Intraocular Lens Dislocation

Atsushi Tohma[1], Yoichiro Masuda[1], Koji Komatsu[1], Ai Iwauchi[2], Hoshiho Kubota[2], Yosuke Nakazawa[3], Tadashi Nakano[1]

[1]Department of Ophthalmology, Jikei University, Tokyo, Japan
[2]Department of Pathology, Jikei University, Tokyo, Japan
[3]Faculty of Pharmacy, Keio University, Tokyo, Japan

Purpose: In Japan, surgeries for intraocular lens (IOL) dislocation have risen ~1.7-fold in the past decade. IOL dislocation results from either zonular weakness, leading to capsular bag dislocation (CBD), or intrinsic capsular fragility, termed dead bag syndrome (DBS). The latter has attracted attention, but its pathogenesis remains unclear. The lens capsule - a basement membrane from lens epithelial cells - is known to accumulate advanced glycation end-products (AGEs) with aging and diabetes. We examined whether AGE accumulation in the lens capsule is associated with IOL dislocation.

Methods: We retrospectively analyzed 10 eyes that underwent surgery for IOL dislocation (DBS, n=5; CBD, n=5) and 11 control eyes that underwent standard cataract surgery at Jikei University Hospital between 2022 and 2024. Excised capsules were immunostained for AGEs, imaged by confocal laser scanning microscopy, and converted to binary images to calculate the AGE-positivity ratio. Groups were compared with the Mann–Whitney U test.

Results: AGE staining was present in all capsules. The mean AGE-positivity ratio was significantly higher in the dislocation group than in controls (15.6 ± 4.9% vs. 4.7 ± 2.7%, $p < 0.001$). Within the dislocation group, DBS showed a higher ratio than CBD (18.5 ± 3.7% vs. 12.6 ± 4.5%, $p = 0.056$), despite the younger age of DBS cases.

Conclusions: AGE accumulation in the anterior lens capsule was markedly greater in IOL dislocation than in controls, with a strong trend in DBS. These findings suggest that AGE deposition may contribute to capsular fragility and delamination, predisposing to IOL dislocation.

Poster 5

The Role of Actin Binding protein, TAGLN, in Myofibroblast Differentiation of Lens Epithelial Cells

Rylee E. King[1], Christian Le[2], Justin Parreno[1,2]

[1]*Department of Biological Sciences, University of Delaware, Newark, DE, USA*
[2]*Department of Biomedical Engineering, University of Delaware, Newark, DE, USA*

Purpose: Ocular lens fibrosis is the result of lens epithelial-to-mesenchymal transition (EMT), during which cells differentiate into highly contractile myofibroblasts. EMT is marked by remodeling of the actin cytoskeleton, including the loss of cortical filamentous (F)-actin and formation of αSMA-enriched stress fibers. Actin remodeling serves as a key regulator of EMT; however, the regulatory molecules that coordinate this remodeling drive myofibroblast differentiation in EMT are not completely understood. Understanding the critical regulators that regulate actin reorganization in EMT may provide new therapeutic insights against lens fibrosis.

Methods: We curated a list of over 50 actin-associated proteins known to be involved in regulating stress fiber formation and evaluated their expression in an *in vivo* surgical mouse model of lens fibrosis using the Lens Injury Response Time Series (LIRTS) RNA-seq dataset. Next, we used an *ex vivo* whole organ culture model to induce lens EMT. We examined the expression of the top candidate gene, Transgelin (Tagln), and its regulation of actin reorganization. F-actin organization and TAGLN localization were assessed by immunofluorescence. Lenses from *Tagln1* knockout mice were used to assess the functional contribution of TAGLN to stress fiber formation.

Results: Forty-three actin-associated genes were upregulated within 120 hours of culture, 12 of which were upregulated within 6 hours post-surgery. *Tagln* was the top upregulated gene at 6 hours. Ex vivo culture led to an upregulation of *Tagln* expression by 6 hours, accompanied by robust stress fiber formation. Whole-mount imaging revealed TAGLN localized to F-actin fibers. Lenses from *Tagln*-null mice showed delayed stress fiber assembly, supporting its role in early EMT regulation.

Conclusions: Our data identify TAGLN as an essential actin-binding protein that promotes the formation of stress fibers during myofibroblast differentiation. The *ex vivo* lens system provides a model for assessing cytoskeletal remodeling in lens fibrosis and will provide insight to determine critical actin binding proteins that may serve as a potential target for therapeutic intervention in lens EMT.

Poster 6

Afadin Orchestrates Tricellular Adherens Junctions and Lens Tissue Architecture

Anna Lubertozzi[1], Angad Dhillon[2], Jessica Martin[2], Timothy Plageman[2]

[1]*Department of Molecular Genetics, Ohio State University, Columbus, OH, USA*
[2]*College of Optometry, Ohio State University, Columbus, OH, USA*

Purpose: The vertebrate lens exhibits a highly ordered arrangement of elongated fiber cells organized into a hexagonal lattice. While this organization has been well described, the mechanisms that generate the pattern remain unclear. Tricellular adherens junctions (tAJs), where three cells meet, have important roles in other epithelia, including distributing mechanical forces and reinforcing adhesion. Examining tAJs in the lens could provide insight into how fiber cell architecture is established. Here, we investigated the molecular composition of lens tAJs and the function of the actin-binding protein afadin in fiber cell organization.

Methods: Immunofluorescence staining of mouse lenses was used to assess junctional protein localization. We identified candidate tAJ components and focused on afadin due to its localization during the onset of fiber cell lattice formation. Afadin function was analyzed using a conditional knockout (cKO) model generated with the lens specific MLR10-Cre line. Morphological, histological, and molecular analyses were performed to assess eye size, lens transparency, fiber cell organization, and protein recruitment.

Results: Screening of adherens junction associated proteins revealed a subset of proteins enriched at tAJs, including afadin, delta-catenin, p120-catenin, ZO-1, and SORBS1. Afadin localized to tricellular junctions after postnatal day 2, coinciding with onset of fiber cell lattice organization. Afadin cKO eyes developed microphthalmia, lenses exhibited opacities, severe disruption of fiber cell elongation, and loss of the regular honeycomb lattice. In addition, other tAJ proteins failed to localize properly in the absence of afadin, suggesting that afadin is necessary for their localization and for tAJ stability.

Conclusions: These findings identify afadin as an essential component of tAJ maintenance in the lens. By linking the actin cytoskeleton to adhesion complexes, afadin promotes fiber cell elongation and organization, as well as lens transparency. This study highlights tAJs as critical regulators of epithelial architecture in the vertebrate lens.

Poster 7

Arvcf stabilizes adherens junctions and Ankyrin-B protein within the lens fiber cell membrane

Electra Coffman[1] and Timothy F. Plageman Jr.[1]

[1]*The Ohio State University, College of Optometry, Columbus, OH, USA*

Purpose: Although age is the highest global risk factor for cataract development, how cell structural changes contribute to its progression remains poorly characterized. We previously established that mice lacking *Arvcf*, a gene encoding a cell adhesion protein, develop disrupted adherens junctions (AJs) as soon as 1 month with premature cortical cataracts by 5 months of age. As mice deficient in the cytoskeletal gene *Ank2 (AnkB)* also possess cell adhesive disruptions, we hypothesize that *Arvcf* and *AnkB* function in a similar pathway to maintain lens fiber cell adhesion over age.

Methods: Lens protein lysates from control and *Arvcf*-deficient mice were analyzed for AJ protein expression by fractionation and western blot quantification. To identify important functional components of lens cell AJs, control lenses were immunoprecipitated with an Arvcf antibody and analyzed by liquid chromatography mass spectroscopy (LC-MS). Lenses from mice lacking *Arvcf* or *AnkB* were further studied by immunofluorescent protein localization quantification.

Results: Western blot reveals that loss of *Arvcf* induces a significant and dramatic decrease in the protein expression of N- and E-Cadherin and their catenin binding partners. We further demonstrate that N-Cadherin associates with both *Arvcf* and AnkB. Interestingly, LC-MS reveals that *Arvcf* strongly associates with the AnkB binding partner NrCAM and gap junction proteins. Immunostaining demonstrates that *Arvcf* facilitates the recruitment of AnkB to the short side lens fiber cell junctions by at least one month and that N-Cadherin localizes well to tricellular junctions while AnkB does not. *AnkB* itself is required for maintaining *Arvcf* in the broad side lens fiber cell junctions as soon as E17.5.

Conclusion: These data suggest that Arvcf may function in part to facilitate the association of Ankyrin-B to the AJ complex and vice versa. They also indicate a shared role for Arvcf and AnkB in stabilizing lens fiber AJs and architecture.

Poster 8

Unilateral Pediatric Cataract with a Thinned Crystalline Lens: A Case Report

Yuka Koshiishi[1], Mayumi Nagata[1], Hiroyuki Matsushima[1]

[1]Department of Ophthalmology, Dokkyo Medical University, Tochigi, Japan

Purpose: Pediatric cataract can manifest with diverse morphological abnormalities of the crystalline lens and often presents significant surgical challenges. We report the clinical course and surgical management of a rare case of cataract with a markedly thinned crystalline lens.

Methods: A 14-year-old boy previously diagnosed with cataract at the age of 9 and lost to follow-up presented with decreased visual acuity. The best-corrected visual acuity (BCVA) of the right eye was counting fingers at 30 cm, and the intraocular pressure was 20 mmHg. Slit-lamp examination of the right eye showed central calcification of the lens capsule with peripheral cortical opacity and scant lens nucleus, leading to the diagnosis of cataract. Cataract extraction was performed on the right eye, and despite the calcified material between the anterior and posterior capsules, the intraocular lens (IOL) was successfully implanted within the capsular bag. Postoperatively, an Nd:YAG laser posterior capsulotomy was performed for residual posterior capsule opacity, and the BCVA improved to 20/50 in the right eye.

Results: In cases of lens–posterior capsule adhesion, surgeons should carefully consider refraining from primary posterior continuous curvilinear capsulorhexis (PCCC) to avoid potential complications of anterior vitrectomy.

Conclusions: This case highlights a rare presentation of pediatric cataract and provides further insight into its surgical treatment.

Poster 9

A Case of Lamellar Separation of the Lens Capsule During Cataract Surgery

Mayumi Nagata[1], Koichiro Mukai[1], Hiroyuki Matsushima[1], Tatsukata Kawagoe[1]

[1]Department of Ophthalmology, Dokkyo Medical University, Tochigi, Japan

Purpose: To report a case of intraoperative lamellar separation of the lens capsule during cataract surgery and to examine the ultrastructural characteristics of the anterior capsule.

Case Presentation: An 83-year-old woman presented with bilateral cataract. Best-corrected visual acuity was 0.15 LogMAR in both eyes. The axial lengths measured 21.50 mm and 21.52 mm in the right and left eyes, respectively, indicating short eyes, and both showed narrow anterior chamber angles. Phacoemulsification and intraocular lens implantation were performed in the right eye. After a 2.4-mm corneoscleral incision, the soft-shell technique and trypan blue staining were used before performing continuous curvilinear capsulorhexis. During hydrodissection, insertion of the viscoelastic cannula was difficult. After re-staining, a membrane-like layer resembling an anterior capsule was observed on the capsulorhexis margin, necessitating a second capsulotomy.

Methods: The excised anterior capsule was fixed in 2.5% phosphate-buffered glutaraldehyde, postfixed in 2% osmium tetroxide, dehydrated in ethanol, and embedded in Epon 812 resin. Semithin (1 μm) sections were stained with toluidine blue for light microscopy, and ultrathin (60 nm) sections were stained with uranyl acetate and lead citrate for transmission electron microscopy.

Results: Electron microscopy revealed alternating layers of high and low electron density on the anterior surface, forming parallel lamellar structures throughout the capsule. The capsule had separated horizontally along these lamellae. The lens epithelial cells on the cortical side appeared normal. Based on these findings, the condition was diagnosed as true exfoliation syndrome.

Conclusions: In true exfoliation, structural fragility of the anterior capsule may lead to intraoperative lamellar separation; therefore, careful surgical manipulation under adequate visualization is essential.

Poster 10

Cataract Survey in an Epidemiological Study on the Health Effects of Occupational Radiation Exposure: Risk Assessment of Three Main Cataract Types by Radiation Dose

Natsuko Hatsusaka[1], Huan Hu[2], Makoto Sasaki[1], Eri Kuboe[1], Toshiaki Okubo[2], Hiroshi Sasaki[1]

1Department of Ophthalmology, Kanazawa Medical University, Ishikawa, Japan
[2]National Institute of Occupational Safety and Health, Japan (JNIOSH), Japan Organization of Occupational Health and Safety, Tokyo, Japan

Purpose: To investigate the association between radiation exposure and three main cataract types—cortical (COR), nuclear (NUC), and posterior subcapsular (PSC)—among emergency workers following the Fukushima Daiichi Nuclear Power Plant accident.

Methods: Ophthalmologic examinations were conducted at 71 clinics, primarily using dilated pupil exams by certified ophthalmologists. Cataracts were classified per the WHO grading system. Participants were screened every three years, with approximately 2,300 individuals examined between 2018 and 2023. Radiation exposure was assessed using effective dose (mSv): cumulative dose before the accident (pre-2011), dose during the emergency work period (apporoximately1 year), and total cumulative dose. Logistic regression adjusted for age was used to evaluate risk.

Results: A total of 2,230 participants (mean age: 58.0 ± 9.5 years) were analyzed. For those examined twice, the second assessment was used. Cataract types: COR only (313), NUC only (587), PSC only (21), mixed (525), no cataract (784). Pre-accident dose was significantly associated with PSC (OR=1.11, 95% CI: 1.03–1.20). Emergency work dose was linked to COR (OR=1.07, 95% CI: 1.01–1.14). Total cumulative dose was associated with COR (OR=1.04, 95% CI: 1.00–1.07) and PSC (OR=1.08, 95% CI: 1.01–1.16). No significant associations were found for NUC or mixed types.

Conclusions: Radiation exposure was significantly associated with increased risk of COR and PSC. Even low-dose exposure may contribute to cataract development through cumulative effects.

Poster 11

Exploration of Hydrophilic Materials Less Prone to Calcium Deposition

Koichiro Mukai[1], Hiroyuki Matsushima[1], Hidetoshi Iwamoto[1], Mayumi Nagata[1], Tatsukata Kawagoe[1]

[1]Department of Ophthalmology, Dokkyo Medical University, Tgochigi, Japan

Purpose: Hydrophilic materials are used as intraocular lens (IOL) materials and offer the advantage of high biocompatibility. However, they are prone to calcium (Ca) deposition as a complication. Focusing on the fact that many different hydrophilic acrylic contact lenses (CLs) are utilized in the contact lens field, we experimentally induced Ca deposition using CLs to explore materials that are less prone to Ca deposition.

Methods: A Mini Sub Cell GT horizontal electrophoresis system (Bio-Rad Laboratories Inc.) was used for the electrophoresis apparatus. The target CLs were Medallist Plus® (water content 38.6%, Bausch & Lomb) made from HEMA (2-Hydroxyethyl methacrylate), Proclear® (water content 60%, Cooper Vision) made from HEMA and MPC (Phosphorylcholine), and 1-Day Acuvue® (water content 58%, Johnson & Johnson) made from HEMA and MAA (Methyl Methacrylate). Each CL was mounted in a dedicated holder with a double-wall structure with circular holes (N=3). The electrophoresis chamber was filled with 10 mM Tris buffer solution prepared by adding 10 mM $CaCl_2$ to the cathode side and 10 mM Na_2HPO_4 to the anode side. The system was operated under conditions of 100V, 25mA, and 40W for 20 hours. After 20 hours, the CLs were removed from the holders and observed under an optical microscope. Von Kossa and Alizarin red S staining were used to evaluate Ca deposited on the CLs.

Results: Crystalline deposits were observed on the surfaces of Medallist Plus® and 1-Day Acuvue® CLs, but no deposits occurred on Proclear®. Von Kossa and Alizarin red S staining showed positive Ca reactions in the deposits, but Proclear® showed no staining.

Conclusions: Among hydrophilic materials, Proclear® made from HEMA and MPC showed no Ca deposition. By improving material composition, it is possible to develop IOLs with high biocompatibility that do not cause calcium deposition.

Poster 12

Pupillary responses in eyes implanted with multifocal and monofocal intraocular lenses

Kohei Miyata[1], Reona Mouri[1], Norihiro Watanabe[1], Natsuko Shima[1], Shinichiro Kobayakawa[1]

[1]Department of Ophthalmology Nippon Medical School Musashikosugi Hospital, Kanagawa, Japan

Purpose: To retrospectively investigate pupillary responses in eyes implanted with a multifocal or a monofocal intraocular lens (IOL), and to examine the correlations between dynamic pupillary parameters (DPPs) and postoperative visual acuity (VA), contrast sensitivity (CS), and vision-related dysphotopsias (VD).

Methods: The multifocal IOL group included 73 eyes of 73 patients implanted with the Synergy® (S group). The monofocal IOL group included 33 eyes of 33 patients implanted with the Vivinex Impress® (V group); in bilateral cases, only the right eye was analyzed. DPPs were measured preoperatively and at 1 month postoperatively using a pupillometer (PLR-3000). Uncorrected distance, intermediate, and near VA were evaluated, and CS and VD were also assessed.

Results: After surgery, both the S group and the V group showed significant decreases in the percentage of pupillary constriction (DELTA), average constriction velocity (ACV), maximum constriction velocity (MCV), and average dilation velocity (ADV) compared with preoperative values. In the S group, DELTA was significantly negatively correlated with uncorrected intermediate VA (UIVA), while in the V group, DELTA was significantly positively correlated with uncorrected distance VA (UDVA). Regarding CS, the V group demonstrated significant positive correlations between DELTA and CS at 1.6, 1.0, and 0.7 degrees. Furthermore, in the S group, a significant positive correlation was observed between the end pupillary diameter (END) and Halo, whereas in the V group, END was significantly positively correlated with Waxy vision.

Conclusions: DPPs change after implantation of both multifocal and monofocal IOLs.DPPs are correlated with uncorrected VA, CS, and VD.

Poster 13

Dynamic Pupillary Responses in Multifocal Intraocular lens (Synergy® and Odyssey®) Implanted Eyes

Norihiro Watanabe

Purpose: Synergy® (J&J) is a diffractive multifocal intraocular lens (M-IOL), while Odyssey® (J&J) is an extended-depth-of-focus (EDOF) IOL using a freeform diffractive design. Odyssey® is the next-generation M-IOL of Synergy®, and both have different optical concepts. We investigated dynamic pupillary responses and visual function (visual acuity and contrast sensitivity) in eyes implanted with Synergy® or Odyssey®.

Methods: A total of 73 eyes implanted with Synergy® and 19 eyes with Odyssey® were examined one month postoperatively. Dynamic pupillary response parameters (DPPs), including constriction ratio (DELTA), average constriction velocity (ACV), and maximum constriction velocity (MCV), were measured using the PLR-3000 (NeurOptics, CA, U.S.A.). Uncorrected distance, intermediate, and near visual acuity, as well as contrast sensitivity, were evaluated. Correlations between DPPs and visual performance were analyzed.

Results: In the Synergy® group, uncorrected near visual acuity showed significant negative correlations with DELTA, ACV, and MCV ($p<0.05$), indicating worse near vision with faster constriction. Contrast sensitivity correlated positively with pupil diameter (INIT and END), suggesting that pupil size may influence optical energy distribution and contrast sensitivity.

In the Odyssey® group, near visual acuity also showed a significant negative correlation with MCV ($p<0.05$), but contrast sensitivity showed no correlation with DPPs. Odyssey® optical design may contribute to its reduced pupil dependency, because it is designed to minimize contrast sensitivity loss through a smoother light-energy distribution.

Conclusions: In the Synergy® group, constriction velocity and pupil diameter were associated with near visual acuity and contrast sensitivity. In contrast, Odyssey® showed a correlation only between constriction velocity and near visual acuity, but not with contrast sensitivity. These findings suggest that differences in optical design may affect dynamic pupillary response and visual function.

Poster 14

Dynamic Pupillary Response and Accommodation with Intraocular Lenses and Crystalline Lenses

Natsuko Maeda[1], Reona Mouri[1], Norihiro Watanabe[1], Kohei Miyata[1], Shinichiro Kobayakawa[1]

[1]Department of Ophthalmology, Nippon Medical School Musashi Kosugi Hospital, Kanagawa, Japan

Purpose: The near response consists of three factors: convergence, accommodation, and pupillary constriction. Convergence and accommodation interact mutually, while near miosis compensates for the decline of accommodative power by increasing the depth of focus. This study investigated dynamic pupillary responses, accommodative response, and the high-frequency component (HFC) in intraocular lens (IOL) eyes and crystalline lenses.

Methods: Subjects included 42 eyes under 45 years without ocular disease (Under45), 38 eyes over 46 years (Over46), 27 eyes with multifocal IOLs under 60 (M-Under60), and 28 eyes with multifocal IOLs over 61 (M-Over61). Accommodative response and HFC, related to ciliary muscle movement, were measured using ACOMOREF2 (Right Mfg. Co., Ltd.). Dynamic pupillary response parameters (DPPs) were evaluated using a dynamic pupillometer (PLR-3000, NeurOptics).

Results: Mean ages were 34.7±7.1 (Under45), 55.6±5.2 (Over46), 47.7±13.0 (M-Under60), and 67.1±3.3 (M-Over61). Mean HFC was 62.8±4.8 in Under45 and 56.5±3.7 in Over46, showing a significant difference ($p<0.05$). HFC was 62.0±8 in M-Under60 and 62.1±5.8 in M-Over61, with no difference. In Under45, accommodative response correlated negatively with pupil diameter and positively with constriction velocity. In M-Under60, HFC correlated negatively with pupil diameter and positively with constriction velocity. No significant correlations were found in M-Over61.

Conclusions: These results suggest that reduced accommodative power with aging may be compensated by pupillary constriction in crystalline lens eyes under 45. Furthermore, interaction between ciliary muscle and dynamic pupillary response may contribute to accommodation in multifocal IOL eyes under 60.

Poster 15

Correlation with Lens accommodation parameters and Retinal age

Reona Mouri[1]

[1]*Nippon Medical School Musashikosugi Hospital, Kanagawa, Japan*

Purpose: We investigated the accommodative response, and the high frequency component (HFC) in intraocular lenses (IOLs) eyes and crystalline lenses using by ACOMOREF2 (Right Mfg. Co., Ltd.). As a result, those lens accommodation parameters (accommodative response and HFC) correlated with chronological age.

On the other hand, the Japan Ocular Imaging Registry (JOIR) had made it possible to estimate retinal age from fundus photographs. In this study, we investigated the correlation with lens accommodation parameters and retinal age, and with chronological age.

Methods: The subjects were 116 eyes (21-85 years old). Retinal age was estimated from fundus photographs using by JOIR pre-training model. Accommodative response and HFC (lens accommodation parameters) were examined using the ACOMOREF2, also investigated the correlation.

Results: A regression equation was obtained between retinal age using JOIR model and chronological age, a regression analysis was performed. Retinal age tended to indicate older than actual age. It was particularly observed in younger age, while to indicate younger than actual in older age.

Furthermore, a negative significant correlation between retinal age and HFC was observed in the group under 60 years old ($p < 0.01$). On the other hand, no significant correlation between retinal age and accommodative response.

Conclusions: We already reported that accommodation parameters (accommodative response and HFC) decreased with aging, especially decreased sharply around 45 years old (accommodative response) and 60 years old (HFC).

These results suggested that aging for retina and ciliary muscle function may progress simultaneously.

Poster 16

Cluster Analysis Identifying High-Risk Biometric Combinations for IOL Calculation Error in High Myopia

Yurui Pan[1,2,3,4], Wenwen He[1,2,3,4], Jiao Qi[1,2,3,4], Jiaqi Meng[1,2,3,4], Keke Zhang[1,2,3,4], Kaiwen Zheng[1,2,3,4], Xiangjia Zhu[1,2,3,4]

[1]Department of Ophthalmology, Eye & ENT Hospital of Fudan University, Shanghai, China
[2]NHC Key laboratory of Myopia and Related Eye Diseases; Key Laboratory of Myopia and Related Eye Diseases, Chinese Academy of Medical Sciences, Shanghai, China
[3]Key Laboratory of Myopia, Chinese Academy of Medical Science, Shanghai, China
[4]Shanghai Key Laboratory of Visual lmpairment and Restoration, Shanghai, China

Purpose: To create a data-driven classification model for highly myopic cataractous eyes and thus identify biometric combinations with high risks of prediction error.

Methods: A total of 23667 highly myopic eyes were used as the clustering dataset. Three clustering algorithms (k-means, kernel k-means, and HDBSCAN) were employed to cluster these eyes based on seven biometric variables: axial length(AL), mean keratometry(Km), lens thickness(LT), white-to-white distance (WTW), corneal astigmatism(CA), the lens thickness to anterior segment ratio and the average corneal curvature to axial length ratio. A postoperative dataset of 511 highly myopic eyes was subsequently divided into subgroups by assigning each eye to its nearest cluster centroid based on Euclidean distance. We then compared the prediction errors of the subgroups with six formulas: Barrett Universal II, Cooke K6, EVO 2.0, Hoffer QST, Kane and Pearl-DGS to identify high-risk biometric combinations.

Results: Five replicable and distinct biometric subgroups were consistently identified by our clustering model. Postoperative error analysis revealed significant differences among these clusters (χ^2=14.02, p=0.016). Notably, cluster 3 — characterized by the highest CA — consistently yielded the highest prediction errors across all six formulas (MedAE=1.07D). Cluster 2 — featuring considerably lower AL, Km and LT — exhibited the second-highest prediction errors (MedAE=0.41D). Cluster 4 — defined by the unique combination of the lowest AL and Km — demonstrated the most accurate predictions (MedAE=0.30D).

Conclusions: Our novel clustering model identified high-risk biometric combinations in highly myopic eyes that can be used to improve the accuracy of IOL calculation.

Poster 17

Postoperative Outcomes of Cataract Surgery with Intraoperative Mydriasis in Eyes with Primary Angles-closure

Soyoka Goto[1], Mayumi Nagata[1], Hiroyuki Matsushima[1], Tatsukata Kawagoe[1]

[1]*Department of Ophthalmology, Dokkyo Medical University, Tochigi, Japan*

Purpose: To evaluate the safety and efficacy of cataract surgery with intraoperative mydriasis in eyes with primary angle closure (PAC).

Methods: Twenty-seven eyes with PAC and 11 open-angle eyes with Mydrin-P® allergy (controls) underwent surgery. Diluted tropicamide/phenylephrine was injected into the anterior chamber, followed by phacoemulsification and IOL implantation. Corrected distance visual acuity (CDVA), intraocular pressure (IOP), endothelial cell loss (ECL), prediction error (SE), and anterior chamber depth (ACD) were assessed preoperatively and at 1 week, 1 month, and 3 months.

Results: CDVA improved significantly in both groups, from 1 week in controls and from 3 months in PAC eyes. IOP decreased significantly at all postoperative visits in PAC eyes. No significant intergroup differences were observed in CDVA, IOP, or SE. At 3 months, ECL was greater in the PAC group (10.5 ± 10.6%, $p = 0.04$). ACD increased significantly in both groups.

Conclusions: Intraoperative mydriasis during cataract surgery was safe in PAC eyes, preventing perioperative IOP elevation and achieving favorable outcomes.

Poster 18

Pax6-K91R Mutant Mice Display Changed Transcriptome and Disrupted Lens Differentiation as Revealed by Single Cell RNA Sequencing (scRNA-seq) Study

Yuyan Shan[1], Shu-Yu Zheng[1], Yu-Wen Gan[1], David W Li[1]

[1]State Key Laboratory of Ophthalmology, Zhongshan Ophthalmic Center, Sun Yat-Sen University, Guangzhou, China

Purpose: Pax6 is indispensable for lens development. We previously showed that SUMOylation at its K91 residue of p32 Pax6 is critical for Pax6 function. The Pax6-K91R mutation leads to severe lens abnormalities and cataract in mice. Here, we characterized how the Pax6-K91R mutation impacts the heterogeneous landscape of lens epithelial cells during their differentiation into fiber cells using scRNA-seq strategy.

Methods: Lens epithelial cells were isolated from P0 wild-type and Pax6-K91R mouse lenses and profiled on the 10x Genomics Chromium platform. Cell Ranger and Seurat were used for QC/integration, clustering, and differential expression. Differentiation trajectories were inferred with Monocle2. The perturbation sensitivity was prioritized by Augur. qPCR and IF on neonatal lenses were used to validate scRNA-seq results.

Results: scRNA-seq of P0 wild-type and Pax6-K91R lenses yielded 16313 high-quality lens epithelial cells. Unsupervised analysis resolved five subpopulations along the epithelial-to-fiber continuum: proliferative progenitors in the germinative zone, early differentiating cells, mid-stage transitional cells, late differentiating fiber-lineage cells, and central-zone epithelium. These subpopulations mapped a continuous differentiation trajectory from progenitor cells to mature fiber cells. In K91R lenses, progenitor and early differentiating cells were expanded, while late fiber-lineage cells were depleted. Perturbation analysis revealed the late differentiating cluster and a stall before terminal states. Concordantly, several families of genes critical for transparency were extensively downregulated.

Conclusions: SUMOylation of Pax6 at K91 acts as a molecular switch enabling terminal lens differentiation. Its loss traps epithelial cells in immature states, leading to significant expression suppression of several families of genes and thereby cataractogenesis. This single-cell atlas pinpoints lens subpopulations and suggests SUMOlation pathway components may be potential therapeutic targets.

Supported by National Natural Science Foundation of China (Grants #82271071, #81970787 & #81770910)

Poster 19

LOXL1 Loss Strengthens Ciliary Zonules and Alters Lens Epithelium Transcriptome

Pooja Rathaur[1], Juan Rodriguez[1,2], Sohini Rebba[1], Philip Ruzycki[1], Steven Bassnett[1]

[1]Ophthalmology & Visual Sciences, Washington University, School of Medicine, St. Louis, MO, USA
[2]University of Health Sciences and Pharmacy, St. Louis, MO, USA

Purpose: The ciliary zonule, a network of extracellular fibers, suspends the lens and facilitates accommodation by transmitting forces from the ciliary muscle. Lysyl oxidase-like 1 (LOXL1) is a crucial cross-linking enzyme within the zonule proteome. Genetic variants in *LOXL1* are strongly associated with exfoliation glaucoma in humans, a condition marked by fragile zonular fibers and the accumulation of abnormal fibrillar material in the anterior segment. To investigate the role of LOXL1 in anterior segment integrity, we examined molecular and functional changes in *Loxl1*-deficient mice.

Methods: LOXL1 knockout mice (*Loxl1*$^{-/-}$) were generated on a C57BL/6 background. Zonular tensile strength was assessed using a customized pull-up assay. In situ hybridization was used to localize *Loxl1* expression in the mouse eye. The size and number of zonular fibers were determined by immunofluorescence imaging. Bulk RNA sequencing of lens epithelial cells was used to assess transcriptomic alterations associated with *Loxl1* loss.

Results: Loss of LOXL1 led to age-dependent strengthening of the ciliary zonule. At one month of age, the zonule of *Loxl1*-null mice was similar to controls. However, by four months, *Loxl1*-null mice exhibited increased zonular tensile strength, which reached statistical significance by six months. Confocal imaging demonstrating enhanced synthesis of fibrillin-1 (FBN1), a key zonular component. Bulk RNA sequencing of *Loxl1*$^{-/-}$ lens epithelia revealed upregulation of set of zonule-associated genes, including *Fbn1*, fibrillin-2 (*Fbn2*), and fibulin-5 (*Fbln5*). These findings suggest that the absence of LOXL1 enhances zonular strength through transcriptional upregulation of extracellular matrix genes.

Conclusions: The unexpected age-dependent strengthening of the zonule in *Loxl1*-null mice implies that the cross-linking enzyme LOXL1 may not play a novel role in regulating the expression of zonule-associated genes.

Poster 20

ANGPTL7 Elevation Drives Intraocular Pressure Dysregulation in Highly Myopic Cataractous Eyes: A Multi-omics Insight

Yu Du[1], Mengchao Zhu[1], Xiangjia Zhu[1]

[1]Department of Ophthalmology, Eye & ENT Hospital, Fudan University, Shanghai, China

Purpose: This study aimed to identify aqueous humor (AH) biomarkers associated with intraocular pressure (IOP) dysregulation in highly myopic cataractous (HMC) eyes using an integrative multi-omics approach, with a focus on angiopoietin like 7 (ANGPTL7) as a potential mediator of post-cataract surgery IOP complications.

Methods: We performed Olink proteomic analysis on AH samples (92 proteins) and bulk mRNA sequencing on lens capsules from HMC eyes and age-related cataract (ARC) as control. Cross-omics validation identified consistently upregulated targets. AH level of the target protein was further quantified via ELISA in a larger cohort (24 HMC vs. 19 ARC). Correlations with baseline IOP and postoperative IOP spikes were assessed. Mechanistic validation was conducted in a defocus-induced highly myopic mouse model using qPCR and Western blot.

Results: Proteomics revealed 10 upregulated proteins in HMC AH, with ANGPTL7 uniquely elevated in both AH and lens transcriptomes. Clinically, AH ANGPTL7 levels positively correlated with baseline IOP (R^2 = 0.324, p = 0.0019) and postoperative IOP spikes (R^2 = 0.2201, p = 0.0136) exclusively in HMC patients. ROC analysis showed strong predictive value for spikes (AUC = 0.921). In highly myopic mice, ANGPTL7 was overexpressed in the trabecular meshwork, coinciding with extracellular matrix remodeling and co-regulation with METRNL.

Conclusions: ANGPTL7 is a key biomarker and mechanistic mediator of IOP dysregulation in HMC eyes, linked to aqueous outflow resistance via trabecular meshwork remodeling. These findings support its potential for surgical risk stratification and targeted therapeutic intervention in HMC patients.

Poster 21

Effects of astaxanthin intake on adiponectin level in human aqueous humor

Hirotaka Hashimoto[1], Kiyomi Arai[1], Eiki Oshida[2], Jiro Takahashi[3], Makoto Chikuda[2]

[1]Tsukuba Hashimoto Optical Clinic, Tsukuba, Ibaraki, Japan
[2]Department of Ophthalmology, Dokkyo Medical University Saitama Medical Center, Koshigaya, Saitama, Japan
[3]Fuji Chemical Industry Co., LTD. Nananiikawa, Toyama, Japan

Purpose and Methods: To explore effects of astaxanthin (AX) intake on adiponectin (APN) level in aqueous humor, aqueous humor samples were taken from 35 patients during cataract surgery which was conducted before and after AX intake (6 mg), and APN level was measured. Also, oxygenation related parameters including superoxide (SO) scavenging activity, H2O2 level, and total hyperoxides (TH) level as well as VEGF level were measured. Then, relationships between APN level (before and after the AX intake) and these parameters, presence of diabetes (DM), age, and sex were evaluated. For the evaluation, multiple regression analysis (stepwise analysis) was used.

Results: There was no significant difference in APN level before and after AX intake. Factors which significantly related to APN level before AX intake were 1) SO scavenging activity, 2) DM, and 3) TH level, and those after AX intake were 1) TH level, 2) DM, 3) sex, and 4) VEGF level, in order of significance.

Conclusions: AX intake does not affect APN level in aqueous humor but would influence the relationship between APN level and SO scavenging activity. Since APN level fluctuated in relation to DM and TH, it was suggested that APN would be involved in DM and peroxidation reactions.

Poster 22

The Protein Phosphatase 1 Regulator, MYPT1 Control Lens Development through Direct Modulation of Functions of HMGA1 and Other Downstream Targets

Xue-Bin Hu*, Xingmiao Liang, David W Li

Purpose: Protein phosphorylation and dephosphorylation play an important role in modulating functions of over 30% total eukaryotic proteins. In lens, we have demonstrated that both PP-1 and PP-2A–mediated dephosphorylation of major transcription factors, and also important signaling components, including AKT and EZH2, have important impact in lens development and pathogenesis. Here, we present evidence to show that the regulatory subunit of PP1, MYPT1 acts as an important regulator to control lens development.

Methods: CRISPRA/Cas9 technology was used to generate MYPT1 knockout zebrafish, RNAseq was used to analyze transcriptomes in both wild type and mypt1$^{-/-}$ zebrafish, phosphor-proteomics was used to identify MYPT1 targets, QRT-PCR and Western blots analysis were used to analyze gene expression, transmission electron microscopy was used to analyze the lens structure. Injection of HMGA1 mRNA into fertilized zebrafish eggs were used to rescue the gene knockout functions.

Results: Silence of MYPT1 gene retards lens differentiation and also causes microphthalmia. RNAseq analysis revealed that expression of eye development related genes including major transcription factors were altered, and the downstream differentiating genes including the 3 families of crystallin genes were significantly downregulated. Phospho-proteomics analysis demonstrated that silence of MYPT1 induces upregulation of the phosphorylation of the HMGA1. Co-immunoprecipitation revealed that MYPT1 directly interacts with HMGA1, indicating direct dephosphorylation of HMGA1 by MYPT1/PP1. Silence of HMGA1 leads to abnormality in lens differentiation which is also associated with altered expression patterns of the related genes found in *mypt1*$^{-/-}$ zebrafish, and such changes in crystallin genes can be reversed with overexpression of HMGA1 mRNA.

Conclusions: Our results reveal that MYPT1 regulates lens differentiation and pathogenesis through HMGA1 and other targets in lens.

Poster 23

Heat Shock Protein 90β Modulates Lens Inflammatory Response to Prevent Cataractogenesis via Direct Interaction with NF-κB p65

Jing-Miao Wang[1], David Wan-Cheng Li[1]

[1]*State Key Laboratory of Ophthalmology, Zhongshan Ophthalmic Center, Sun Yat-Sen University, Guangzhou, Guangdong, China*

Purpose: Heat shock protein 90β (HSP90β) is a key molecular chaperone implicated in diverse cellular processes. Although it prevents aging-related cataract formation through CHMP4B and p53 **(Fu et al., 2023, PNAS)**, its role in regulating the lens microenvironment is unclear. In this study, using various molecular and cellular biotechnologies, we provide evidence that HSP90β can directly interact with nuclear factor-κB (NF-κB) p65 and thereby modulate the inflammatory response within the ocular lens.

Methods: HSP90β was knocked out or knocked down in FHL124 and αTN4-1 cells (LECs) using CRISPR/Cas9 and shRNA and suppressed in zebrafish with morpholinos. RNA-seq and qRT-PCR were used to profile inflammatory cytokine expression; Western blotting and immune-fluorescence were used to assess NF-κB components; Co-IP and immunofluorescence confirmed HSP90β–p65 interaction. Zebrafish animal models were used to evaluate p65's role in regulating lens development.

Results: Both in lens epithelial cells and zebrafish animal models, downregulation of HSP90β expression led to a significant increase in the expression levels of inflammatory cytokines, accompanied by elevated expression and activity of the NF-κB. Immunofluorescence staining and Co-IP s further confirmed that HSP90β modulates the inflammatory response in LECs through direct interaction with the p65 subunit of NF-κB. Furthermore, overexpression of p65 in zebrafish resulted in cataractogenesis and microphthalmia, which were concurrently associated with increased apoptosis of LECs.

Conclusions: Collectively, our findings demonstrate that HSP90β plays a fundamental role in maintaining lens homeostasis, with a critical function in suppressing inflammatory responses within the lens. Mechanistically, it does so through direct interaction with the NF-κB p65 to regulate the downstream signaling cascade.

Supported by National Natural Science Foundation of China (Grants #82271071, #81970787 & #81770910)

Poster 24

Effects of Tenascin-X Deficiency on Anterior Segment Morphology

Yuka Okada[1], Takayoshi Sumioka[2], Shingo Yasuda[2], Ai Matsushita[2], Hiroki Iwanishi[2], Masayasu Miyajima[2], Sanyog Polkam[3], Matthew A Reilly[3], Cynthia J Roberts[3], Ken-Ichi Matsumoto[4], Shizuya Saika[2]

[1]Department of Ophthalmology, Wakayama Medical University Kihoku Hospital, Wakayama, Japan.
[2]Department of Ophthalmology, Wakayama Medical University, Wakayama, Japan.
[3] Department of Ophthalmology & Visual Sciences, College of Medicine, The Ohio State University Wexner Medical Center, Columbus, Ohio, USA
[4]Shimane University, Interdisciplinary Center for Science Research, Department of Biosignaling and Radioisotope Experiment, Shimane, Japan

Purpose: Tenascin-X (TNX) is one of the causative genes of Ehlers–Danlos syndrome (EDS) and acts as a matricellular protein regulating collagen fibrillogenesis. Ocular abnormalities such as keratoconus and high myopia have been reported in EDS patients. This study aimed to investigate the functional role of TNX in the cornea and lens using TNX-knockout (KO) mice.

Methods: Thirty-two C57BL/6 wild-type (WT) and thirty-two KO mice were used.

(1) TNX expression was examined by immunostaining in WT embryos at embryonic days 14.5 and 15.5. (2) Corneal thickness and anterior chamber depth (ACD) were measured by anterior segment OCT and compared between groups. After imaging, corneal diameter, axial length, and cornea/axial length ratio were determined. (3) Lens height was defined as the distance from the line connecting both scleral spurs to the anterior lens apex on OCT images and compared between groups; cataract progression was also evaluated. (4) Zonular fibers were immunostained for fibrillin-1, and RNA extracted from ocular tissues was analyzed for fibrillin-1 expression by real-time RT-PCR.

Results: （1） During embryogenesis, TNX was detected in the corneal epithelium, stroma, and ciliary region. （2） Corneal thickness was significantly greater and ACD deeper in KO mice, whereas axial length and corneal diameter showed no difference. At 2 years, KO mice exhibited a lower cornea/axial length ratio. （3） Lens height was significantly lower in KO mice, suggesting posterior lens displacement. Cataracts were more advanced in KO mice. （4） Immunostaining showed no marked difference in fibrillin-1 localization; however, RT-PCR revealed reduced fibrillin-1 expression in KO corneas.

Conclusions: Loss of TNX may contribute to increased corneal thickness, zonular fragility, posterior lens displacement, and cataract progression.

Poster 25

Lens sclerosis is promoted by Piezo1 channel through transglutaminase 2 activation

Yosuke Nakazawa[1], Yuri Doki[1], Rosica S. Petrova[2], Noriaki Nagai[3], Paul J. Donaldson[2], Megumi Funakoshi-Tago[1]

[1]*Faculty of Pharmacy, Keio University, Tokyo, Japan*
[2]*School of Medical Sciences, University of Auckland, Auckland, New Zealand*
[3]*Faculty of Pharmacy, Kindai University, Osaka, Japan*

Purpose: Presbyopia is caused by age-related lens hardening, resulting in near vision loss, and it occurs in almost every individual aged ≥50 years. Piezo1 is a mechanosensitive channel that constantly senses pressure changes during the regulation of visual acuity. This study aimed to elucidate the relevance of Piezo1 activation and cross-linking in the development of presbyopia.

Methods: Frozen sections were prepared from the lenses of 9-week-old C57BL/6 mice. Cross-linking of γ-crystallin was examined by immunoblot analysis. Cell lysates were prepared and electrophoresed on a 15% polyacrylamide gel. The gel was transferred to a PVDF membrane and reacted with antibodies. Yoda1 (5 μM) was instilled into the eyes of 9-week-old C57BL/ 6JJ mice three times daily for 15 days. After 15 days, the lens was extracted, and the lens was measured to calculate lens hardness

Results: Activation of Piezo1 by Yoda1 significantly increased TGM2 mRNA expression and enzymatic activity, mediated by ERK1/2 and JNK1/2 pathways. Ex vivo lens treatment demonstrated that Yoda1 promoted γ-crystallin cross-linking through TGM2 activation. Furthermore, in vivo administration of Yoda1 eye drops induced lenticular hardening via TGM2 upregulation and activation, suggesting that Piezo1 activity is directly linked to lens sclerosis.

Conclusions: These findings demonstrate that Piezo1 activation promotes lens stiffening through TGM2 induction and protein cross-linking, providing novel insight into the mechanisms underlying presbyopia. Importantly, Yoda1-treated mice represent a promising animal model of presbyopia, suitable for screening pharmacological agents targeting lens stiffening. This model may facilitate the development of new therapeutic strategies to prevent or delay presbyopia progression.

Wednesday, Dec. 10th

Wednesday, December 10th

Session 8: Redox Biology & Cataract

Moderators: *Julie Lim & Xingjun Fan*

Talks are in the format of 12 minutes presentation with 3 minutes discussion.

Time	Speaker	Title
8:30 am	**Xingjun Fan**	Lipid Peroxidation Increases Lens Stiffness and Plasma Membrane αA-Crystallin Association
8:45 am	**Hongli Wu**	Redox imbalance triggers lens inflammaging and senescence: insights from Grx1/Grx2 double-knockout mice
9:00 am	**Kim Jiang**	Investigating the molecular mechanisms involved in cataract formation post vitrectomy
9:15 am	**Yu Pu**	Removal of N-terminal cysteines from γD-crystallin generates aggregation-prone dimers
9:30 am	**Vincent Monnier**	In silico methods for improving binding affinities of gamma crystallin aggregation inhibitors
9:45 am.	**Julie Lim**	Unlocking the clock: bringing to “light” the mechanisms involved in antioxidant regulation in the lens

Lipid Peroxidation Increases Lens Stiffness and Plasma Membrane αA-Crystallin Association

Xingjun Fan[1], Emily Fan[2], Caili Hao[1], Kazi Radeen[1], Zongbo Wei[1]

[1]*Department of Cellular Biology and Anatomy, Medical College of Georgia at Augusta University, Augusta, GA, USA*
[2]*Department of Biological Sciences, Franklin College of Arts and Sciences, University of Georgia, Athens, GA, USA*

Purpose: Age-related lens stiffening is a hallmark of presbyopia and is closely linked to oxidative stress. Oxidative insults promote lipid peroxidation, altering the physicochemical properties of the fiber cell plasma membrane and potentially influencing cytoskeletal anchoring and crystallin–membrane interactions. However, the contribution of lipid peroxidation–driven membrane adaptation to lens stiffness and protein aggregation remains poorly defined. This study used an ex vivo mouse lens culture model to determine how lipid peroxidation impacts lens biomechanics and αA-crystallin membrane association.

Methods: Lenses from two-month-old mice were cultured ex vivo with or without the GPX4 inhibitor RSL3, the glutathione synthesis inhibitor buthionine sulfoximine (BSO), or the glutathione-chelating compound dimethyl fumarate (DMF). Lens stiffness was quantified using a MicroTester LT mechanical analyzer. Membrane-bound αA-crystallin was assessed by immunoblotting of isolated membrane fractions, and protein aggregation was quantified by dynamic light scattering.

Results: Inhibition of GPX4 activity or depletion of lenticular glutathione markedly increased lipid peroxidation, as indicated by elevated 4-HNE adducts. Sustained lipid peroxidation significantly enhanced lens stiffness and increased αA-crystallin association with the plasma membrane. These biochemical changes were accompanied by a pronounced rise in high-molecular-weight protein aggregates detected by light-scattering analysis.

Conclusions: These findings demonstrate that lipid peroxidation is a potent biomechanical modifier of the lens, linking oxidative stress to age-related stiffening and crystallin aggregation. Enhanced αA-crystallin binding to peroxidized membranes may represent an adaptive, but ultimately saturable, mechanism for preserving membrane integrity during oxidative stress. Progressive accumulation of lipid peroxidation products likely contributes to biomechanical dysfunction and cataractogenesis in the aging lens.

Redox imbalance triggers lens inflammaging and senescence: insights from Grx1/Grx2 double-knockout mice

Hongli Wu[1,2], Ying Qin[1], Yu Yu[1], Taegun Kwon[3], Janak Sunuwar[3]

[1]Department of Pharmaceutical Sciences, University of North Texas Health Science Center at Fort Worth, Fort Worth, TX, USA
[2]North Texas Eye Research Institute, University of North Texas Health Science Center at Fort Worth, Fort Worth, TX, USA
[3]Research Core Labs, University of North Texas Health Science Center at Fort Worth, Fort Worth, TX, USA

Purpose: Oxidative stress is a key driver of lens aging and cataract formation, but its role in initiating chronic inflammatory signaling within this immune-privileged tissue is poorly understood. We hypothesized that loss of the redox regulators glutaredoxin-1/2 (Grx1/Grx2) accelerates lens epithelial cell (LEC) senescence and triggers a senescence-associated secretory phenotype (SASP) that promotes cataractogenesis.

Methods: Primary LECs were isolated from wild-type (WT) and Grx1/Grx2 double-knockout (DKO) mice at 1 and 12 months (n=3/group). Senescence was assessed by SA-β-gal staining and cell-cycle markers (p53, phospho-RB). Bulk RNA-seq with differential expression and pathway enrichment analyses profiled inflammation and SASP programs; selected targets (e.g., IL-6) were validated by qPCR and immunoblot. To test therapeutic modulation, LECs were treated ± metformin and senescence quantified.

Results: DKO LECs exhibited clear evidence of premature and accelerated senescence, characterized by early onset and a heightened degree of cellular aging compared with age-matched WT controls. SA-β-gal positivity was evident in 1-month DKO and increased markedly at 12 months versus age-matched WT. DKO LECs showed elevated p53 and reduced phospho-RB, consistent with arrest. Transcriptomic profiling revealed broad dysregulation with significant enrichment of inflammatory signaling (interleukin, NF-κB and cytokine-mediated pathways) and SASP components. IL-6 was increased at mRNA and protein levels in aged LECs and was highest in 12-month DKO. Metformin reduced cellular senescence in a dose-dependent manner in both WT and DKO cultures.

Conclusions: Redox imbalance from Grx1/Grx2 loss establishes an intrinsic inflammaging program in the lens independent of classical immune-cell infiltration. Coupled senescence (↑p53, ↓phospho-RB) and SASP activation (↑IL-6) link oxidative stress to lens aging and cataract initiation. Together, these findings establish Grx1/Grx2 DKO mice as an ideal model to study redox-driven inflammatory cataract mechanisms and highlight senescence-targeted interventions, such as metformin, as promising strategies to delay lens opacity progression.

Commercial Relationships: *Hongli Wu, None; Ying Qin, None; Haoxin Liu, None; Yu Yu, None; Taegun Kwon, None; Janak Sunuwar, None*

Support: *Grant No. 1R21EY033941–01A1 to Wu*

Investigating the molecular mechanisms involved in cataract formation post vitrectomy

Jiang L[1,2], Martis RM[1,2], Pan X[1,2], Bar-David L[2,3], Rotstein T[2,3], Donaldson PJ[1,2], Lim JC [1,2]

[1]Department of Physiology, School of Medical Sciences, University of Auckland, Auckland New Zealand
[2]New Zealand National Eye Centre, University of Auckland, Auckland, New Zealand
[3]Department of Ophthalmology, School of Medical Sciences, University of Auckland, Auckland, New Zealand

Purpose: Vitrectomy is one of the most commonly performed ocular procedures in which the vitreous humour is removed to access and treat retinal disorders and then replaced with saline. Although vitrectomy is essential for the treatment of multiple retinal disorders, an unfortunate consequence of vitrectomy is that patients will develop a cataract within one to two years. The underlying molecular mechanisms remain poorly understood. To address this, we developed a rabbit model to investigate the molecular and biochemical factors contributing to post-vitrectomy cataract development.

Methods: Eye examinations were conducted on 6-month male or female NZ white rabbits to assess ocular health and magnetic resonance imaging (MRI) to measure oxygen levels and lens free water content. Vitrectomy was subsequently performed on the right eye, with the left eye serving as a control. Following an 8-week recovery period, post-surgical changes to lens transparency, lens free water content and oxygen levels were assessed. Rabbits were then euthanised and ocular tissues collected for biochemical analysis of antioxidants and oxidative stress markers.

Results: 8 weeks following vitrectomy, lenses were transparent. However, in the vitreous, an increase in oxygen levels and a reduction in ascorbate concentrations ($p<0.0001$) was found in the vitreous chamber of the vitrectomy eye relative to control. There was a significant decrease in lens water content in the posterior side of the lens of the vitrectomy eye relative to control ($p=0.03$), while antioxidant levels and oxidative stress markers in the lens remained similar to control.

Conclusions: While there were early signs of changes to the vitreous, there were no major effects on lens health. It is likely that over time the changes observed in the vitreous, will impact the lens eventuating in cataract formation enabling this model to be used for testing of therapies to delay the onset of cataract post vitrectomy.

Removal of N-terminal cysteines from γD-crystallin generates aggregation-prone dimers

Yu Pu[1,2,3], Vanie Seecharan[4], Aslam Uddin[2,3], Eugene Serebryany[1,2,3,5]

[1]*Department of Chemistry*
[2]*Department of Physiology and Biophysics*
[3]*The Laufer Center for Physical and Quantitative Biology*
[4]*Department of Pharmacology*
[5]*The Stony Brook Cancer Center, Stony Brook University, Stony Brook, NY, USA*

Purpose: Aging lenses become increasingly oxidizing, allowing non-native disulfides to drive aggregation of γ-crystallins. Despite this vulnerability to disulfides, γ-crystallins evolved to be Cys-rich. To understand this paradox, we investigated how mutating all four Cys residues in the N-terminal domain of human γD-crystallin affects its stability and aggregation. We hypothesized that Cys removal would prevent the disulfide-driven aggregation pathway we reported previously but would impose a separate fitness cost.

Methods: We expressed and purified human γD-crystallin (WT) and its two N-terminal cysteine substitution variants. The NCS variant (C18S/C32S/C41S/C78S) replaces Cys with Ser, considered the most conservative substitution. The NCA/T variant (C18T/C32A/C41A/C78A) replaces Cys with the more rigid and hydrophobic Thr and Ala instead. We measured stability by equilibrium and kinetic denaturation in guanidinium (Gdn) at 37°C, monitored by intrinsic tryptophan fluorescence. We used solution turbidity to quantify the aggregation rate of monomeric and dimeric proteins as a function of [denaturant], [protein], and temperature. We also characterized the novel aggregates' morphology by transmission electron microscopy.

Results: Both variants exhibited reduced stability compared to WT and a biphasic curve (melting of the N-terminal domain followed by melting of the C-terminal domain) Both variants aggregated without needing oxidizing agents. Maximal aggregation rate was achieved at [Gdn] near the midpoint of the first equilibrium unfolding transition. Surprisingly, the aggregation precursor was a native-like dimer held together by a C-terminal disulfide. Aggregation rate depended linearly on the concentration of this dimer, suggesting that misfolding within the dimer molecule was the rate-limiting step for aggregation.

Conclusions: The N-terminal Cys residues contribute substantially to native-state stability in human γD- crystallin. Their removal prevents aggregation via non-native disulfides but promotes aggregation via conformational rearrangement of native-like disulfide-bridged dimers. Future studies may investigate whether a similar conformational rearrangement can take place in the native or post-translationally modified wild-type γD-crystallin.

In silico methods for improving binding affinities of gamma crystallin aggregation inhibitors

Vincent M Monnier[1,2], Eunsoo Cho[1], Michael Do[1]

[1]Department of Pathology and
[2]Biochemistry, Case Western Reserve University, Cleveland, OH, USA

Purpose: Gamma crystallins are essential for lens transparency. They aggregate and lose transparency when thermally stressed, oxidized, deamidated, glycated or mutated. We previously reported that thermal aggregation of Human or Mouse γS and γD is prevented by Closantel (Drug C) and other small molecules (Islam et al JBC 2022). While C did not prevent cataract in the OPJ F9S-γS mutant mouse, it significantly improved lens opacity (pub. pend). Drug C acted as a chaperone mimetic by binding to identical residues as the αA "MAC" peptide K^{70}-K^{88}. The relatively low binding affinities for C to relevant sites in HgD and HgS (-6.3 to -8.4 kcal/mol) led us to test two approaches for improving binding affinities.

Methods: Method A: systematic swapping of functional groups of drug C and determination of binding affinities to HγD (1HK0 ; PDB) and MγS (1ZWN; PDBe) using Autodock Vina3. Method B: Using Virtual Flow open source platform https://doi.org/10.1038/s41586-020-2117-z for automated AI-driven docking of 1.4 billion compounds onto 1HK0 and selecting binding affinities better than -8.4 kcal/mol.

Results: Method A: optimization of Closantel with "isobutylamide 4-aminobenzamide + carboxylated C-3/C-5 + stripped Ring C" resulted in increased affinities to HγD of -9.3 (up from 8.4 kcal/mol) at the N-C tail spanning domain. Method B: after two months of computing time, 7 compounds with binding affinities between -12 and -13 kcal/mol (SMINA) were obtained. The most promising compound is a thiocarbamate with N-C-Tail binding activity resembling Closantel with Autovina binding affinity of -9.5 kcal/mol and interactions with S39, C41, R59....R169, R172, S174, thus expected to stabilize both the HγD N and C domains.

Conclusions: Both chemical structure manipulation of Closantel and AI-driven search resulted in finding candidate drugs with improved binding affinities to the crucial NC-binding domain of HγD crystallin.

Unlocking the clock: bringing to “light” the mechanisms involved in antioxidant regulation in the lens

Julie C Lim[1,2], Fenger Ou Yang[1,2], Paul J Donaldson[1,2], Raewyn C Poulsen[3] & Yadi Chen[1,2]

[1]Department of Physiology, School of Medical Sciences, University of Auckland, Auckland, New Zealand
[2]Aotearoa New Zealand National Eye Centre, University of Auckland, Auckland, New Zealand
[3]Department of Pharmacology, University of Auckland, Auckland, New Zealand

Purpose: In previous work, we have shown that cultured lenses maintain time of day differences in the expression of circadian clock and redox genes under 12-hour light/12-hour dark conditions. However, when lenses were cultured under constant darkness for 24 hours, time-of-day difference in clock and redox genes were not maintained, suggesting that light acts as an important cue for maintaining clock and redox gene expression in the lens. Recently, we localised the blue light sensitive protein melanopsin to the central epithelium suggesting that the lens may be able to detect light directly.

Methods: Melanopsin activation in the lens was tested by calcium imaging. Rat lenses were loaded with CAL590-AM calcium dye and then placed in a recording chamber. Blue light stimulation at the central epithelium, where melanopsin is present, or equatorial epithelium stimulation, where melanopsin is absent, was performed using Laser Scanning Microscopy stimulation and time-lapse images were captured every 3 seconds to monitor changes in calcium levels. Following imaging, fluorescence intensity values from four selected regions of interest was extracted. Fluorescence traces were normalised to baseline values and changes in calcium signal before and after blue light stimulation were analysed using GraphPad Prism software.

Results: Blue light exposure durations (2 seconds, 5 seconds, 10 seconds, 20 seconds, 2 minutes, and 6 minutes) and laser intensities (0.55%, 2%, 4%, 6%, 8%, and 10%) were tested for calcium responses. A sustained increase in calcium levels was observed when the central epithelium, but not the equatorial epithelium, was stimulated for 6 minutes under low intensity (0.55%) conditions.

Conclusions: Melanopsin appears to be functional in the rat lens suggesting that the lens can detect and respond to light. Future work will involve determining whether this calcium mediated response activates circadian clock proteins to mediate antioxidant regulation in the lens.

Wednesday, December 10th

Session 9: Lens Optics & Biomechanics

Moderators: *Barbara Pierscionek & Kehao Wang*

Talks are in the format of 15 minutes presentation with 3 minutes discussion.

Time	Speaker	Title
10:30 am	**Barbara Pierscionek**	Reflections on the optics of the lens
10:48 am	**Ji He**	Individual Variation of Refractive Index of the Crystalline Lens of the Normal and Cataract Eyes
11:06 am	**Qing Li**	Retrieving the gradient refractive index structure of the crystalline lens from the wavefront aberrations over two-dimensional visual field
11:24 am	**Alyssa Lie**	An in vivo MRI study of changes in lens water distribution with cataract development
11:42 am	**Kehao Wang**	Biomechanical simulations reveal key parameters that affect lens accommodation

Reflections on the optics of the lens

Barbara K Pierscionek[1], Kehao Wang[2]

[1]*Faculty of Health, Medicine and Social Care, Medical Technology Research Centre, Anglia Ruskin University, Chelmsford, United Kingdom*
[2]*School of Engineering Medicine, Beihang University, Beijing, China*

Purpose: The optical properties of the lens: shape and refractive index determine its refractive power. The latter property is not easy to measure given its variation across the tissue. A number of techniques have been employed to determine how the refractive index varies with age and will be described.

Methods: Methods of refractive index measurement include those that are invasive or require tissue slicing and those which can be conducted on whole lenses. Each has its limitations as well as strengths. Non-invasive or minimally invasive methods include ray tracing, fibre optic sensing and most recently X-ray Talbot interferometry. A number of other methods that have measured water content have been used to estimate refractive index; these vary in accuracy.

Results: The refractive index variation in the human lens is largely in the cortex with the nucleus exhibiting a relatively constant magnitude of refractive index. This differs from animal lenses that have a more parabolic refractive index profile increasing into the nucleus. The developmental changes in the refractive index also vary across species.

Conclusions: Different measurements of the optical parameter of refractive index provide varying insights and levels of accuracy. There is broad agreement about the general shape of the refractive index though less concurrence about magnitude. The X-ray Talbot interferometric method is thus far the detailed and allows measurement of the 3-dimensional refractive index profile as well as measurement of changes with opacification. Direct measurements of refractive index in vivo have not yet been possible.

Individual Variation of Refractive Index of the Crystalline Lens of the Normal and Cataract Eyes

Ji C. He[1], Jianhua Wang[2]

[1]New England College of Optometry, Boston, MA, USA
[2]Bascom Palmer Eye Institute, University of Miami, Miami, FL, USA

Purpose: Accessing the refractive index of crystalline lenses in the human eye in vivo has long been a challenge. Recently, a ray traceable Scheimpflug imaging (RTSI) equipped anterior segment optical coherence tomography (AS-OCT) system has been successfully developed to measure the lens refractive index *in vivo*. This study aimed to measure the lens refractive index for a group of normal and cataract patients by using the new technology.

Methods: The combined AS-OCT and RTSI system sharing the same scanning light source (about 850nm) was used to imaging the right eyes of 15 normal subjects and 10 cataract patients. The images were processed to trace the light ray from the cornea to the lens and to solve the lens refractive index by using a self-developed MatLab program. The average lens refractive index along the optical axis (N_{av}) were derived.

Results: The lens N_{av} varies from 1.38 to 1.43 between the normal subjects and cataract patients. The lens N_{av} tended to increase with age and also tended to be increased for the cataract patients as compared with the normal subjects. Cataract patients had lens Nav varied substantially from individual eye to individual eye.

Conclusions: Ray-traceable Scheimpflug imaging-equipped AS-OCT is useful for testing the refractive index of lenses in the human eye *in vivo*. The lens refractive index varies substantially from individual to individual, not only for the normal eyes but also for the cataract patients. Influence of the lens refractive index on the measurement of axial length of the eye and hence the IOL power calculation should be estimated during cataract surgery planning.

Retrieving the gradient refractive index structure of the crystalline lens from the wavefront aberrations over two-dimensional visual field

Qing Li[1], Bing Xie[1]

[1]Ruijin Hospital, School of Medicine, Shanghai Jiao Tong University, Shanghai, China

Purpose: In vivo measurement of the gradient index distribution (GRIN) of the crystalline lens is crucial for building the individual eye model. This work proposes a new method of retrieving the GRIN lens based on the wavefront aberrations over the central and peripheral visual field.

Methods: A deep-learning based modelling procedure is proposed to establish the relationship between the wavefront aberrations of the crystalline lens over multiple field angles and the crystalline lens model parameters. As a starting point, the physiology-like lens model from our previous work is applied to generate tens of thousands of computational lens models, providing the training data as groups of 13 physiology-based lens parameters, visual field angles and the corresponding Zernike coefficients of wavefront aberration. The relationship between the error of prediction, the complexity of the lens model and the number of the visual fields has been investigated systematically by ray tracing and manipulation of multiple deep-learning based models.

Results: The initial model based on multi-layer perception is built by training 90% of the 30,000 groups of lens data, with the rest data for model testing. Normalization has been applied to the input and output parameters. With 500 steps of iteration, the model could predict the lens parameters with high accuracy. Once the deep-learning based modelling procedure has been built, the prediction of the lens parameters cost a few ms. The error of prediction decreases with increasing number of visual field angles. The average error reaches below 5% with three visual field for a rotational-symmetric lens model.

Conclusions: A new deep-learning based model has been built to retrieve the lens GRIN structure from axial and peripheral wavefront aberrations with high accuracy and efficiency. The prediction error of the model can be further decreased with the addition of aberrations over more visual field locations.

An in vivo MRI study of changes in lens water distribution with cataract development

Alyssa L. Lie[1,2], Xingzheng Pan[1], Thomas W. White[3], Paul J. Donaldson[1]

[1]Department of Physiology, School of Medical Sciences, University of Auckland, Auckland, New Zealand
[2]School of Optometry and Vision Science, University of Auckland, Auckland, New Zealand
[3]Department of Physiology and Biophysics, SUNY at Stony Brook, Stony Brook, NY, USA

Purpose: Cataract is the leading cause of preventable blindness worldwide. Lens transparency is the outcome of several cellular adaptations that minimize light scattering, which are in turn supported by a unique microcirculation system that regulates the internal water distribution of the lens. In this study we employed magnetic resonance imaging (MRI) to test our hypothesis that disruption of the steady-state lens water distribution contributes to cataract development.

Methods: 22 adults (12 male, 10 female) aged between 58 and 84 years (mean ± SD = 71 ± 7 years) underwent MRI on a 3T clinical scanner (MAGNETOM Vida Fit; Siemens, Germany) to acquire two-dimensional T1 maps. T1 values representing free water content were then extracted using custom MATLAB routines. Axial distributions of free water across the anterior and posterior lens were independently characterized by a power function of normalized radial distance from the lens center. Cataract severity was graded in accordance with the Lens Opacification Classification System (LOCS III).

Results: As nuclear cataract is the most strongly associated with aging, only LOCS III nuclear opalescence (NO) and color (NC) scores were considered. A moderate positive correlation was found between nuclear cataract severity and age ($r = 0.568$, $p = 0.006$). After controlling for age, nuclear cataract severity showed a moderate negative correlation ($r = -0.438$) with the central lens free water content that approached statistical significance ($p = 0.054$).

Conclusions: The data suggest nuclear cataract is associated with reduced free water in the central lens, possibly reflecting protein aggregation in this region. This contradicts the increase in central free water content we previously observed in non-cataractous aged lenses. The difference in directional change suggests cataractogenesis involves processes additional or distinct to aging and indicates that cataract may be prevented or delayed through treatments targeted at maintaining physiological homeostasis in the lens.

Biomechanical simulations reveal key parameters that affect lens accommodation

Kehao Wang[1], Barbara K Pierscionek[2]

[1]School of Engineering Medicine, Beihang University, Beijing, China
[2]Faculty of Health, Medicine and Social Care, Medical Technology Research Centre, Anglia Ruskin University, Chelmsford, United Kingdom

Purpose: During the process of accommodation, the lens shape is modulated by the force transmitted from the ciliary muscle by the zonules. The mechanism of accommodative loss with age has not yet been fully understood because the complex mechanical behaviour of the lens-zonules-ciliary body system is hard to measure in vivo. Here we develop Finite Element (FE) models that show physiological relevance to investigate the changes in lens accommodative behaviour with age.

Methods: A series of human eye lens models were developed based on optical measurements taken from biological lenses of different ages ranging from 16 to 62 years old at the synchrotron imaging facility (SPring-8) in Japan. The influence of material properties, zonular angles and zonular stretching mechanisms were investigated. Variations in distributions of cortical elastic moduli were considered such that a gradient distribution of elastic moduli that resembled the distribution of refractive index was created. A range of different combinations of zonular angles and the individual functions of different zonular groups were also considered by coupling the lens model to the whole eye.

Results: Results show that models with gradient elastic moduli have similar deformation behaviour and optical response as models with uniform elastic moduli. The zonular angles can greatly affect the optical response with the combination of anterior, equatorial and posterior zonular angle of [10°, 0°, -24°] provided the maximal change in optical power. In addition, the anterior and equatorial zonules were found to play vital roles during accommodation. Among these parameters, the lens geometric changes with age show the major influence on lens optical power.

Conclusions: These simulation results suggest that lens accommodation is a complex multi-factious process. Variations in material distribution mainly affect lens stress distribution, zonular fibres and lens geometries both affect the lens deformative and optical change.

Thursday, December 11th

Thursday, December 11th

Session 10: Lens Membranes & Physiology

Moderators: *Tom White & Paul Donaldson*

Talks are in the format of 15 minutes presentation with 3 minutes discussion.

Time	Speaker	Title
8:30 am	**Jean Jiang**	Dual-domain Regulation of Connexin 50 Suppresses Lens EMT and Post-surgical Fibrosis
8:48 am	**Taishi Painter**	Role of Slc20a2 in Lens Growth and Fiber Cell Differentiation and Elongation
9:06 am	**Yadi Chen**	Regional differences in Ca^{2+} mobilization mediated by zonular tension in the rat lens
9:24 am	**Nikhail Nair**	Dissecting the contributions of AQPs to regional lens water homeostasis: insights from lens nanovesicle and hydrostatic pressure analysis
9:42 am	**Nick Delamere**	A comparison of hydrostatic pressure responses to Piezo-1 and TRPV4 in the mouse lens

Dual-domain Regulation of Connexin 50 Suppresses Lens EMT and Post-surgical Fibrosis

Bo Ma[1], Zhaodan Ding[1], Guangyan Wang[1], Sumin Gu[1], Jean X. Jiang[1]

[1]Department of Biochemistry and Structural Biology, University of Texas Health Science Center, San Antonio, TX, USA

Purpose: Posterior capsule opacification (PCO) is the most common cause of secondary vision loss after cataract surgery, driven by lens epithelial cell epithelial–mesenchymal transition (EMT). The molecular mechanisms maintaining epithelial integrity remain incompletely understood. Here, we identify a critical role of Connexin 50 (Cx50) in preserving lens epithelial phenotype and suppressing PCO progression.

Methods: We used in vitro, ex vivo, and in vivo models, including primary chick lens epithelial cells, lens organ cultures, and $Cx50^{-/-}$ mice subjected to extracapsular lens extraction. The effects of TGF-β2 on Cx50 expression and EMT induction were examined by immunostaining, Western blotting, and qPCR for α-smooth muscle actin (α-SMA), fibronectin, and E-cadherin. Cx50 overexpression and dominant-negative E2-domain mutants were used to assess domain-specific functions.

Results: TGF-β2 downregulated Cx50 and induced α-SMA and fibronectin, effects reversed by Cx50 overexpression. Mechanistically, the E2 domain of Cx50 mediates intercellular adhesion, limiting epithelial migration and EMT, while the C-terminal domain stabilizes β-catenin–E-cadherin complexes, preventing TGF-β2–driven E-cadherin loss and β-catenin nuclear translocation. Dominant-negative adhesion deficient E2 mutants abolished these effects, and $Cx50^{-/-}$ lenses exhibited enhanced EMT and fibrosis following surgery.

Conclusions: Cx50 preserves epithelial homeostasis through a dual-domain mechanism integrating cell adhesion and adherens junction stabilization. These findings suggest Cx50 as a novel regulator of lens epithelial integrity and a promising target to prevent fibrotic PCO and other post-surgical complications.

Role of Slc20a2 in Lens Growth and Fiber Cell Differentiation and Elongation

Taishi Painter[1], Chun-hong Xia[1], Xiaohua Gong[1]

[1]School of Optometry and Vision Science Program, University of California, Berkeley, CA, USA

Purpose: To investigate how Slc20a2 deficiency affects lens growth during embryonic and postnatal development by analyzing lens epithelial and fiber cell morphology in Slc20a2 knockout (Slc20a2KO) mice.

Methods: Lenses from wild-type (WT), heterozygous, and homozygous Slc20a2KO mice at multiple ages were examined by cryo-sectioning and immunofluorescence. Epithelial cell proliferation was assessed using EdU labeling, and apoptosis was evaluated by TUNEL assay.

Results: At embryonic day 15.5 (E15.5), Slc20a2KO lenses showed no significant changes in fiber cell morphology compared to wild-type (WT) controls. However, immunostaining at postnatal day 2 (P2) revealed fiber cell elongation defects in KO lenses, including misoriented apical tips in differentiating fiber cells and disruption of the lens fulcrum. At P2, Slc20a2KO lenses displayed greater variability in lens diameter (0.899±0.101 mm; n=20) compared to WT lenses (0.981±0.038 mm; n=16). EdU labeling at P2 also showed increased variance among KO samples, reflecting two distinct subgroups: approximately 21.4% of KO lenses showed little to no EdU labeling, while the rest were comparable to WT. TUNEL staining at P14 detected no apoptosis in Slc02a2KO lens epithelial or differentiating fiber cells.

Conclusions: These results demonstrate that Slc20a2 is essential for proper lens development during the early postnatal period. The disruption of differentiating fiber cells and the lens fulcrum–together with the absence of apoptosis and the lack of a consistent reduction in epithelial proliferation–suggests that impaired fiber cell elongation is the primary cause of reduced lens size in Slc20a2KO mice. Altogether, this data shows how Slc20a2 helps maintain the fiber cell structure necessary for normal lens size, and its loss leads to variable growth defects resulting in a smaller lens in knockout mice.

Regional differences in Ca^{2+} mobilization mediated by zonular tension in the rat lens

Yadi Chen[1,2], Paul J Donaldson[1,2]

[1]Department of Physiology, School of Medical Sciences, University of Auckland, Auckland, New Zealand
[2]Aotearoa New Zealand National Eye Centre, University of Auckland, Auckland, New Zealand

Purpose: To investigate how changes in zonular tension are transduced into region-specific Ca^{2+} signalling via mechanosensitive channels in the intact lens using established confocal imaging methods.

Methods: Rat lenses with intact zonules were imaged using confocal microscopy. Epithelial cells were loaded with Cal520 AM, while fiber cells were microinjected with Fluo-8 pentapotassium salt. Zonular tension was modulated pharmacologically using pilocarpine (to decrease tension) or tropicamide (to increase tension). To test dependence on zonular tension and extracellular calcium, experiments were repeated after cutting the zonules or in calcium-free artificial aqueous humor (AAH). The role of TRPV channels was examined using HC-067047 (TRPV4 inhibitor) and A-889425 (TRPV1 inhibitor).

Results: Confocal imaging revealed distinct Ca^{2+} responses in central and equatorial epithelial cells and differentiating fiber cells. Tropicamide, which increases zonular tension, evoked a delayed but transient Ca^{2+} rise in both epithelial regions. In the equatorial epithelium, this response required intact zonules and extracellular Ca^{2+}, as it was abolished after zonule cutting or in Ca^{2+}-free conditions. In the central epithelium, the response was only partially suppressed, suggesting additional mechanisms of Ca^{2+} release. Pilocarpine, which decreases zonular tension, failed to elicit measurable epithelial responses. In contrast, fiber cells showed robust and sustained Ca^{2+} influx to both drugs, dependent on intact zonules and extracellular Ca^{2+}. Pretreatment with TRPV1 (A-88) or TRPV4 (HC-06) inhibitors did not alter Ca^{2+} responses in epithelial cells. In fiber cells, TRPV4 inhibition reduced the tropicamide-induced Ca^{2+} rise but did not abolish it, while TRPV1 inhibition produced a smaller Ca^{2+} increase that remained elevated after washout with AAH.

Conclusions: Our novel tissue preparation and imaging protocols allows us to investigate how changes in zonular tension are transduced via mechanosensitive channels into changes in Ca2+ that activate cell signalling pathways known to control lens water transport and therefore the optical properties of lens.

Dissecting the contributions of AQPs to regional lens water homeostasis: insights from lens nanovesicle and hydrostatic pressure analysis

Nair N[1], Donaldson P[1], Vorontsova I[1], Petrova R[1], Chen Y[1], Schey K[2]

[1]Department of Physiology, University of Auckland, New Zealand
[2]Department of Biochemistry, Vanderbilt University, TN, USA

Purpose: While several aquaporins (AQP0, AQP3 and AQP5) have been shown to be differentially expressed in lens fiber cells, their regional contributions to water permeability (PH2O) have not been measured. To address this, a novel method for measuring regional PH2O was developed and correlated with assessment of overall lens water transport obtained by measuring lens hydrostatic pressure.

Methods: Native nanovesicles from the cortex and nucleus were prepared from bovine or zebrafish lenses and passed through a stopped-flow apparatus to measure rate of hypertonicity-induced shrinkage by a light scattering assay. The obtained rate constant was used to calculate PH2O. Relative contributions of AQPs to PH2O were tested by using loss-of-function Aquaporin 0 zebrafish mutants or by incubation of bovine lens vesicles with AQP3 and/or AQP5 inhibitors. Intracellular pressure of the WT and Aqp0 mutant zebrafish lens were measured using a modified picospritzer-microelectrode approach.

Results: Higher membrane PH2O was measured in nanovesicles from the lens cortex compared to the nucleus from both bovine and zebrafish. Regional PH2O was inversely proportional to hydrostatic pressure, which was high in the lens nucleus, and low in the cortex. AQP5 and AQP0, but not AQP3 contributed to PH2O in the lens cortex and nucleus. Furthermore, loss-of-function zebrafish Aqp0 mutants reduced lens hydrostatic pressure gradient compared to WT.

Conclusions: While it has been shown that conductivity of gap junctions determines lens hydrostatic pressure gradient, our data also implies a role of AQP0 and AQP5 in local PH2O that is inversely proportional to hydrostatic pressure in the cortex and nucleus. Both AQP0 and AQP5 appear to contribute to lens cortex and nucleus equally. The tightly regulated water transport systems ultimately set up lens refractive index properties, so future studies could target AQPs for the development of novel therapeutic approaches to prevent or treat cataract or refractive error.

A comparison of hydrostatic pressure responses to Piezo-1 and TRPV4 in the mouse lens

Junyuan Gao[2], Richard T Mathias[2], Kunlandiappan Varadaraj[2], Mohammad Shahidullah[1], Jeffrey Mei[1] and Nicholas A Delamere[1]

[1]Department of Physiology, University of Arizona, Tucson, AZ
[2]Department of Physiology & Biophysics, State University of New York, Stony Brook, NY

Purpose: The lens uses mechanosensitive ion channels TRPV4 and TRPV1 in a feedback control system that senses intracellular hydrostatic pressure (HP) fluctuations and adjusts ion transporters to compensate. It also expresses another mechanosensitive channel, Piezo1. The goal of study was to test whether Piezo1 is activated by changes in HP and examine the functional consequences.

Methods: Intracellular HP in mouse lenses was measured with a microelectrode-manometer system.

Results: The Piezo1 agonist Yoda1 transiently reduced HP and recovery required TRPV1. The TRPV4 agonist GSK1016790A (GSK) added after Yoda1 caused an additional HP reduction, suggesting Piezo1 and TRPV4 are independent. Moreover, inactivation of TRPV4 by the antagonist HC067047 did not prevent the Piezo1 response. When HP was elevated by partial Na/K pump inhibition and simultaneous inhibition of TRPV4-dependent recovery, HP remained elevated even though activation of Piezo1 by Yoda1 was able to restore HP to zero. This suggests Piezo1 is not activated by positive HP. The radial HP gradient in the fiber mass was determined in lenses treated with either Yoda1 or GSK after TRPV1 was blocked to prevent HP recovery. In both cases the slope of the HP gradient in the differentiating fiber (DF) region was reduced by ~ 50%. Interestingly, the DF responses to Yoda1 and GSK were absent in lenses subjected to inhibition of tyrosine phosphatase PTP-1B by a selective inhibitor, TCS401.

Conclusions: Piezo1 activation causes a negative change of HP at the lens surface. Piezo1 and TRPV4 operate independently and cause additive pressure reduction responses. Unlike TRPV4, Piezo1 is not activated by HP changes and likely plays no role in feedback control. Pressure gradient responses to Piezo1 or TRPV4 activation suggest possible phosphatase-dependent activation of latent Na/K pumps in DF cells.

Thursday, December 11th

Session 11: Lens Development & Regeneration

Moderators: *Kelly Tseng & Mason Posner*

Talks are in the format of 15 minutes presentation with 3 minutes discussion.

Time	Speaker	Title
10:30 am	**Irene Vorontsova**	Cross-talk between lens power and axial eye length: Unravelling mechanisms leading to myopia
10:48 am	**Hideaki Morishita**	PLAAT phospholipases drive autophagy-independent organelle clearance in the lens for transparency
11:06 am	**Wei Li**	PLCϒ functions in lens vesicle separation by modulating FGF signaling
11:24 am	**Kelly Tseng**	Investigating Developmental Lens Regrowth
11:42 am	**Konstantinos Sousounis**	Cellular states and trajectories during newt lens regeneration

Cross-talk between lens power and axial eye length: Unravelling mechanisms leading to myopia

Irene Vorontsova[1], Aan Chu[2], John Phillips[2], Kehao Wang[3], Barbara K. Pierscionek[4], Paul J. Donaldson[1]

[1]Department of Physiology, School of Medical Sciences, The University of Auckland, New Zealand
[2]School of Optometry and Vision Science, The University of Auckland, New Zealand
[3]Beijing Advanced Innovation Centre for Biomedical Engineering, School of Biological Science and Medical Engineering, Beihang University, Beijing, China
[4]Faculty of Health, Medicine and Social Care, Medical Technology Research Centre, Chelmsford Campus, Anglia Ruskin University, United Kingdom

Purpose: To achieve a functional optical system, the development of lens power must coordinate with the growing eye to focus light onto the retina. While the retina has been shown to play a key role in sensing image blur to adjust eye growth, it is unknown whether and how lens optical development is regulated. This knowledge gap is hindering better understanding of why unregulated eye growth occurs in children leading to myopia (short-sightedness), which has been projected to affect 50% globally by 2050. While myopia can be optically corrected, high myopia (<-6D) increases risk of irreversible blindness urging for more effective preventative strategies. The objective of this study was to test whether cross-talk between lens optics and axial eye length exists using the zebrafish as a model.

Methods: The gradient of refractive index and shape of zebrafish lenses ~2.5 months old were measured by X-ray Talbot interferometry and lens power calculated by optical modelling software Zemax. Calculated focal lengths were compared with eye axial length obtained by SD-OCT. Lens power-eye length cross-talk was challenged using form deprivation by monocular application of opaque fibrin bioadhesive to the cornea to induce axial eye elongation, and aqp0a-/- null mutants were used to increase lens power.

Results: Lens power developed in coordination with WT axial eye length. Lenses with high lens power had shorter axial eye lengths, while form deprived eyes with longer axial length had lower lens power.

Conclusions: We show for the first time that changes in eye axial length led to changes in lens optical power. We also confirmed that changes in lens power leading to changes in axial length to match this power. These findings unravel new mechanisms of cross-talk in the eye that normally lead to an emmetropic system, which can now be developed into new myopia-prevention targets.

PLAAT phospholipases drive autophagy-independent organelle clearance in the lens for transparency

Hideaki Morishita[1], Satoshi Nakahara[1], Fumiya Okawa[1], Kan Etoh[1], Tomoya Eguchi[2], Noboru Mizushima[2]

[1]Department of Molecular Cell Biology, Graduate Seal of Medical Sciences, Kyushu University, Fukuoka, Japan
[2]Department of Biochemistry and Molecular Biology, Graduate School of Medicine, The University of Tokyo, Tokyo, Japan

Purpose: Terminal differentiation of lens fiber cells requires the complete removal of all organelles for transparency, yet the molecular machinery driving this process has remained elusive. We aimed to identify the core mediators and elucidate the mechanism of this non-classical organelle degradation pathway.

Methods: We utilized an in vivo knockout screen in zebrafish to discover essential components. The function of the identified PLAAT family was confirmed in zebrafish and mouse models. Evolutionary analysis was conducted across vertebrates. To spatially resolve the HSF4-dependent mechanism, we employed spatial transcriptomics on lens tissues, followed by functional characterization of the resulting gene candidates.

Results: We identified the highly conserved phospholipase A/acyltransferase (PLAAT) family as indispensable mediators. PLAAT activity was crucial for clearing mitochondria, ER, and lysosomes, and thereby for lens transparency. Mechanistically, cytosolic PLAAT phospholipases translocate to damaged organelle membranes in an HSF4-dependent manner, triggering complete membrane dissolution (Morishita H* et al., *Nature* 2021; Morishita *Curr Opi Physiol* 2022). Evolutionary analysis showed PLAAT is conserved across most vertebrates, but genes were pseudogenized or lost in several sightless, deep-dwelling species, suggesting loss of selection for lens transparency.

Conclusions: These findings define an autophagy-independent, HSF4-dependent organelle degradation pathway mediated by cytosolic phospholipases. The evolutionary loss of PLAAT in sightless vertebrates further supports its specific role in transparency. This mechanism is vital for maintaining lens clarity, and the characterization of new factors from our spatial data is poised to expand our understanding of organelle disposal.

PLCϒ functions in lens vesicle separation by modulating FGF signaling

Wei Li[1], Qian Wang[1]

[1]*John F. Hardestry MD Departments of Ophthalmology and Vision Sciences, Washington University in St. Louis, Saint Louis, MO, USA*

Purpose: Fibroblast Growth Factor (FGF) signaling plays critical roles in lens development. The downstream effectors of FGF signaling include Ras/extracellular signal-regulated kinase (ERK), phosphatidylinositol-3-kinase (PI3K)/AKT and phospholipase C-γ (PLCγ). Although Ras/ERK and PI3K/AKT signaling cascades have been intensively studied, PLCγ's function in FGF signaling is much less understood. Therefore, this study focused on the role of PLCγ in FGF-induced lens development.

Methods: We used a mouse line carrying Y769F mutation in Fgfr2 (*Fgfr2*$^{\Delta PLC\gamma}$), a key binding site for PLCγ (Wang Q, et al., 2025). Conditional knockout mouse models were generated using lens-specific cre (Le-cre). Embryos were harvested and sectioned for immunostaining. Mouseprimary lens epithelial cells were cultured and adenovirus expressing Cre recombinase induced deletion of flox alleles. FGF signaling was detected by western blot and immunostaining.

Results: We found that mutation of the PLCγ binding site on Fgfr2 (*Fgfr2*$^{\Delta PLC\gamma}$) resulted in a persistent lens stalk during mouse embryo development. Immunofluorescence analyses displayed a higher expression of FGFR2 in *Fgfr2*$^{\Delta PLC\gamma}$ mutant lens compared to the wild-type group. Moreover, up-regulation of the FGF downstream targets including AKT, Erk and mTOR signaling was observed in *Fgfr2*$^{\Delta PLC\gamma}$ mutant lens, consistent with the gain-of-function phenotype of FGF signaling. Lens-specific knockout PLCγ1 (*PLCγ1*^{CKO}) recapitulates the lens stalk phenotypes observed in *Fgfr2*$^{\Delta PLC\gamma}$ mutant. These results suggest a negative "brake" role of PLCγ in FGF signaling and a critical regulating role of Fgfr2 in PLCγ activation.

In vitro, we isolated primary lens epithelial cells from *Fgfr2*$^{\Delta PLC\gamma}$ and *PLCγ1*$^{flox/flox}$ lens. We found that FGFR2 expression was preserved at a significantly higher level in *Fgfr2*$^{\Delta PLC\gamma}$ lens epithelial cells and after PLCγ1 deletion, compared to control cells. In addition, the *PLCγ1* deleted lens epithelial cells displayed robust activation of Akt and Erk, which was consistent with the in vivo data.

Conclusions: PLCγ signaling plays a pivotal role in FGF-induced lens vesicle separation. It has a negative regulating effect on FGF downstream signaling to avoid the pathological development of lens vesicle. In further study, whether PLCγ takes actions through regulating the endocytosis and degradation of FGFR2 will be detected.

Investigating Developmental Lens Regrowth

Kelly Tseng[1]

[1] *School of Life Sciences, University of Nevada Las Vegas, Las Vegas, USA*

Purpose: Mammals have a limited ability to regrow organs whereas animals such as frogs can restore lost structures. The clawed frog, Xenopus laevis, is a well-characterized model for both animal development and regeneration studies. Individual Xenopus eye tissues such as the retina and lens can undergo regeneration. Here, we investigate the ability of frog embryos to regrow the lens in a developmental context.

Methods: Eye and lens tissues were ablated surgically, and the lens regrowth process was examined using cellular and molecular methodologies including histology, microscopy, and micro-computed tomography.

Results: Tailbud embryos readily regrew lenses after ablation. Lens and retinal regrowth occurred concurrently. Within five days, the regrown eye reached a similar size as the contralateral uninjured eye and contained the full structures including the lens and retina, connected to the brain, and was functional. To compare the eye optics of the developing and regenerating lenses, the lens nucleus localization pattern was assessed. Similar to zebrafish, the tadpole lens nucleus shifted from an anterior to a central location in the optical axis during the prometamorphosis period. In eyes regenerated after embryonic ablation, tadpole lens nuclei were also anteriorly localized then centralized before metamorphosis, recapitulating the same pattern as control developing eyes. Moreover, lens nuclei localization in optical axes in developing and regenerated Xenopus eyes show close correlations to axial eye length.

Conclusion: These results showed that frog embryos can re-initiate lens formation after ablation. Both developing and regrown lenses nuclei are initially anteriorly localized in optical axes in larvae, then centralize at older stages. Our findings suggest a conserved evolutionary mechanism for eye optical development between Xenopus and zebrafish. This study highlights the lens regrowth model as a robust platform to systematically and efficiently define the mechanisms needed for coordinated eye regeneration.

This study provides a new in vitro model for regeneration study and demonstrates the potential of NAM in in vitro mammalian lens regeneration

Konstantinos Sousounis[1]

[1]*Department of Molecular, Cellular, and Biomedical Sciences, University of New Hampshire, Durham, NH, USA*

Purpose: Newts can regenerate their eye lens throughout life. Following the complete removal of the lens, dorsal-residing iris pigmented epithelial cells (IPECs) transdifferentiate to lens epithelial cells (LECs). The purpose of this study was to characterize gene expression and cellular state changes during IPEC-to-LEC reprogramming.

Methods: scRNAseq was used in Pleurodeles waltl newt irises at ten different timepoints encompassing all major phases of lens regeneration starting from intact iris to lens vesicle formation. In silico analysis was performed using Trailmaker and Monocle3. Dorsal and ventral IPECs were identified using the positional markers TBX5 and VAX2, respectively.

Results: 80,425 cells were analysed and clustered into 13 different iris and associated cell types. The majority of IPECs were found to highly express LTBP2. Subclustering of IPECs from the intact iris sample revealed six putative subpopulations: two dorsal, three ventral, and one with mixed positional identity, indicating that IPECs are heterogeneous before regeneration is initiated. Subclustering of IPECs from all timepoints uncovered 16 clusters representing the different cellular states acquired during regeneration. Gene expression enrichment analysis revealed functions related to melanin biosynthesis, cell cycle, migration, extracellular matrix organization, and cell adhesion. Pseudotime trajectory analysis was highly correlated with the actual timecourse and revealed gene expression changes as MITF+ IPECs reprogram to PROX1+ LECs. Regulatory element enrichment analysis identified putative new reprogramming factors that control different phases of the IPEC-to-LEC conversion.

Conclusions: Our study provides novel insights into gene expression changes and cellular transitions during newt lens regeneration. Characterizing this process could uncover ways to translate it to humans for therapeutic purposes.

Disclaimer: *Research reported here was supported by the NEI/NIH under Award Number R00EY029361. The content is solely the responsibility of the authors and does not necessarily represent the official views of the NIH.*

Thursday, December 11th

Session 12: PCO, Fibrosis and EMT

Moderators: *Janice Walker & Michael Wormstone*

Talks are in the format of 15 minutes presentation with 3 minutes discussion.

Time	Speaker	Title
1:15 pm	**Janice Walker**	Defining the metabolic and bioenergetic requirements that fuel the lens response to injury to promote a fibrotic outcome to wounding
1:33 pm	**Promita Deb**	Role of α5β1 integrin in the pathogenesis of fibrotic posterior capsular opacification (PCO)
1:51 pm	**Lan Zhang**	CREB controls STING-Mediated Signaling of Inflammatory Response of the Ocular Lens through the Transcriptional Repressor REST and STING ISGylation during Lens Fibrosis
2:09 pm	**Qiuli Fu**	Generation and application perspectives of regenerated lens during cataract treatment
2:27 pm	**Michael Wormstone**	Menadione-induced depletion of glutathione (GSH) impairs lens epithelial cell function and viability: A potential strategy for posterior capsule opacification management

Defining the metabolic and bioenergetic requirements that fuel the lens response to injury to promote a fibrotic outcome to wounding

Janice L. Walker[1], Kiril K. Bahadure, Kylie R. Stabler, Erin L. Seifert

[1]Thomas Jefferson University, Philadelphia, PA, USA

The metabolic and bioenergetic requirements involved in navigating a lens injury response toward a fibrotic outcome resulting in posterior capsule opacification (PCO), are not well understood. We investigated the requirement of three key metabolic pathways in driving the pathological response to lens injury: glycolysis, mitochondrial OXPHOS, the major ATP source, and glutaminolysis, which breaks down glutamine to generate TCA cycle metabolites. For these studies, we utilized an ex vivo post-cataract surgery explant model, that mimics cataract surgery, and recapitulates the major features of PCO. Explants were treated with vehicle or inhibitors that block glycolysis or mitochondrial function. Explants were also cultured under metabolically controlled environments, glucose-free or glutamine-free conditions. The impact of treatments on fibrotic marker expression: the myofibroblast marker αSMA and/or fibrotic extracellular matrix, fibronectin (FN) and collagen I (Col I) were determined by immunofluorescence and/or western blot analysis. Treatment with 2-deoxy-D-glucose to block glycolysis showed that glycolysis is required to drive the fibrotic response to lens injury. Culturing explants in glucose-free media with glutamine did not prevent αSMA expression associated with fibrosis. However, explants grown under media conditions containing glucose but free of glutamine partially reduced αSMA expression. Altogether these findings highlight roles for glutaminolysis along with glucose in the transition to a myofibroblast phenotype as well as indicate metabolic plasticity of the cells in modulating the lens injury response. To assess mitochondrial OXPHOS function, explants were treated with oligomycin to inhibit ATP synthase or rotenone, to inhibit complex I. While oligomycin did not affect fibrosis, inhibiting complex I led to reduction of αSMA, FN, and Col I, suggesting a role for mitochondria independent of OXPHOS. Together these findings begin to map the metabolic and bioenergetic requirements needed to induce a fibrotic phenotype in response to lens injury and identify potential new therapeutic targets for treating PCO.

Role of α5β1 integrin in the pathogenesis of fibrotic posterior capsular opacification (PCO)

Promita Deb[1], Suhotro Gorai[1], Imane Assakhi[1], Adam P. Faranda[1], Melinda K. Duncan[1]

[1]Department of Biological Sciences, University of Delaware, Newark, DE, USA

Purpose: Fibrotic PCO requires transforming growth factor-beta (TGF-β) signaling and fibrotic extracellular matrix (ECM) assembly for remnant lens epithelial cells (LEC) to transition into myofibroblasts (EMT). Fibronectin is crucial for both fibrotic ECM assembly and sustained myofibroblast phenotype in an animal model of PCO although the mechanisms are unclear. As LECs increase expression of the canonical fibronectin receptor α5β1 integrin post-lens injury (PLI), we hypothesize that α5β1 integrin is critical for fibrotic PCO pathogenesis.

Methods: Lens specific *Itga5* gene knockout mice (α5cKO; MLR10Cre) were created and evaluated for transparency. RNA sequencing analysis was performed on WT and α5cKO injured LECs at 0 and 48 hours PLI. Data was validated by semi-quantitative immunofluorescence and statistically assessed with Student's t-test, and ANOVA with a secondary multiple comparisons test.

Results: α5cKO lenses remain transparent while maintaining normal refractive function through 8 months, although they are larger than controls and have thicker capsules. Following lens injury, adult α5cKO lenses exhibit an attenuated fibrotic response with attenuated fibrotic marker upregulation, diminished canonical TGF-β signaling as measured by pSMAD2/3 levels, and reduced injury-induced LEC proliferation. α5cKO LECs also show attenuated focal adhesion kinase (FAK) phosphorylation PLI suggested integrin signaling is reduced. Furthermore, α5cKO LECs differentially express many more genes at 48 hours PLI than LECs unable to express its ligand fibronectin (Fn1cKO), including a broader attenuation of fibrotic marker upregulation and sustained inflammatory responses suggesting that α5β1 integrin acts earlier in the injury response than cellular fibronectin produced by LECs.

Conclusions: This study identifies α5β1 integrin as a key driver of the LEC EMT response and suggests that its regulation of FAK signaling is key to this process. Thus, it is possible that therapies disrupting integrin-dependent fibrotic ECM assembly could prevent EMT-related PCO after cataract surgery.

CREB controls STING-Mediated Signaling of Inflammatory Response of the Ocular Lens through the Transcriptional Repressor REST and STING ISGylation during Lens Fibrosis

Lan Zhang[1], Yuwen Gan[1], Jiayu Li[1], David W. Li[1]

[1]The State Key Laboratory of Ophthalmology, Zhangshan Ophthalmic Center, Sun Yat-sen University, Guangzhou, China

Purpose: Increasing studies have revealed that ocular lens is subject to immune surveillance, and that the inflammation response of lens epithelial cells (LECs) contributes to lens fibrosis following cataract surgery. We recently established the dual roles of Wild type CREB and S133A-CREB, a mutant mimicking constant dephosphorylation at S133 in regulating mesenchymal transition of lens epithelial cells (EMT). This study was aim to explore the mechanisms by which WT-CREB and S133A-CREB regulating inflammation signaling during lens fibrosis

Methods: shRNA and gRNA were used to silence gene expression. qPCR, Western Blot, and whole-mount immunofluoresce were used to examine the differential gene expression.

Results: WT-CREB and S133A-CREB have distinct roles regulating inflammation signaling during lens fibrosis. First, WT-CREB co-operates REST to suppress Sting transcription. Either Creb/Rest gene knockout or RE-1 element disruption substantially restored the STING expression in mouse LECs overexpressing WT-CREB. Second, S133A-CREB promotes the ISGylation and the protein stability of STING, thereby functions as a positive factor for STING expression. Third, HERC6 functions as E3 ligase of STING ISGylation in mouse LECs. However, TRIM25, instead of HERC6, serves as the predominant E3 ligase of STING ISGylation in the mouse LECs overexpressing S133A-CREB. Finally, TGFβ-treated-mouse LECs overexpressing S133A-CREB and the lens epithelia of S133A-CREB transgenic mice displayed enhanced expression of Sting and downstream chemokine/cytokines.

Conclusion: We demonstrated that CREB controls STING-mediated signaling of inflammatory response through transcriptional repressor REST and ISGylation during lens fibrosis. Mechanistically, WT-CREB co-operates REST to suppresses STING-mediated chemokine signaling. In contrast, S133A-CREB promotes the STING ISGylation and the expression of downstream chemokine/cytokines.

Generation and application perspectives of regenerated lens during cataract treatment

Qiuli Fu[1,2,3], Yuzhou Gu[1,2,3], Lu Chen[1,2,3], Yuhao Wu[1,2,3], Jiali Yuan[1,2,3], Ke Yao[1,2,3]

[1]Zhejiang University, Eye Center of the Second Affiliated Hospital, School of Medicine, Hangzhou, Zhejiang Province, China
[2]Zhejiang Provincial Key Lab of Ophthalmology, Hangzhou, Zhejiang Province, China
[3]Zhejiang Provincial Clinical Research Center for Eye Diseases, Hangzhou, Zhejiang Province, China

Purpose: In the process of exploring new methods for cataract treatment, lens regeneration is an ideal strategy for effectively restoring accommodative vision and avoiding postoperative complications and has great clinical potential. Lens regeneration, which is not a simple repetition of lens development, depends on the complex regulatory network comprising the FGF, BMP/TGF-β, Notch, and Wnt signaling pathways.

Methods: Experiments were conducted through transcriptome sequencing, qRT-PCR and immunofluorescence on human lenses, lens organoids, rabbit regenerated lenses, mouse lenses, and cell lines.

Results: Our research mainly focuses on in situ and in vitro lens regeneration. On the one hand, the possibility of the autologous stem cell in situ regeneration of functional lenses has been confirmed; on the other hand, both embryonic stem cells and induced pluripotent stem cells have been induced into lentoid bodies in vitro which are similar to the natural lens to a certain extent. Meanwhile, patient-specific lentoid bodies were also served as human-derived disease model for pathological mechanism investigation of cataract.

Conclusions: In summary, our work related to lens regeneration both in situ and in vitro provides reference and possibilities for its clinical transformation.

Menadione-induced depletion of glutathione (GSH) impairs lens epithelial cell function and viability: A potential strategy for posterior capsule opacification management

Michael Wormstone[1,2], Preeya Ganger[2]

[1]Nottingham Ningbo China Beacons of Excellence Research and Innovation Institute, University of Nottingham Ningbo China, Ningbo, China
[2]School of Biological Sciences, University of East Anglia, Norwich, United Kingdom

Purpose: Cataract surgery induces a wound healing response by lens epithelial cells within the remaining lens tissue (capsular bag). Modifications on the posterior capsule can lead to reduced visual quality and is termed posterior capsule opacification (PCO). In many cases, laser surgery is required to correct this problem. Finding solutions to prevent PCO is an important healthcare objective. In the present study we utilised Menadione as a glutathione (GSH) depleting agent and observed the cellular and physiological consequences of its actions.

Methods: The human lens epithelial cell line FHL124 and in vitro human capsular bag model were utilised. The Cell-TitreGlo (CTG) Luminescent assay was employed to determine cell viability. ROS levels were established with a DCFDA cellular ROS assay. Western blot methods were utilised to assess ER stress and autophagy markers. Mitochondrial networks were visualised using Mitotracker Red CMXROS viewed using confocal microscopy. Capsular bags were prepared from human donor eyes obtained with ethical approval by following the key stages of cataract surgery. A ciliary body/zonule capsular bag complex was transferred to a 6 well plate and maintained in 6ml EMEM. Match-paired experiments were performed and cultured for ≥15 days

Results: Application of 10μM Menadione to capsular bag cultures suppressed cell coverage of the central posterior capsule relative to non-treated control. 30μM Menadione treatment resulted in complete cell loss. The same concentration-dependent response pattern on cell viability was observed in FHL124 cells. Menadione treatment (30μM) of FHL124 cells increased ROS levels, disrupted mitochondrial networks, enhanced ER stress and increased autophagy. Treatment with the ROS scavenger NAC, or GSH prevented Menadione-induced cell death, supported mitochondria network integrity, prevented endoplasmic reticulum (ER) stress and limited autophagy.

Conclusions: GSH reduction following Menadione application to lens epithelial cells increases oxidative stress, which perturbs vital cell processes leading to cell death. Menadione is therefore a putative therapeutic agent for the management of PCO following cataract surgery that should be explored further.

Thursday, December 11th

Session 13: Crystalline Lens Optical Evaluation; Inspiration and Guidance for IOLs and Presbyopia

Moderator: *Susana Marcos*

Talks are in the format of 12 minutes presentation with 3 minutes discussion.

Time	Speaker	Title
3:00 pm	**Susana Marcos**	Crystalline lens full 3-D geometry: implications for intraocular lens development and selection
3:15 pm	**Kirill Larin**	Optical Coherence Elastography and Brillouin Spectroscopy of Crystalline Lens
3:30 pm	**Liane Clamen**	Development of an Accommodating Intraocular Lens that Mimics the Young, Human Crystalline Lens
3:45 pm	**Andres de la Hoz**	Finite element models of accommodation for estimation of crystalline lens material properties
4:00 pm	**Alberto de Castro**	Optical coherence tomography to study the gradient index of refraction of the human crystalline lens
4:15 pm	**Eduardo Martinez-Enriquez**	Eigenlens geometrical representation of the crystalline Lens

Crystalline lens full 3-D geometry: implications for intraocular lens development and selection

Susana Marcos[1,2,3,4], Eduardo Martínez-Enríquez[4], Alberto de Castro[4], Douglas Koch[5], Mujdat Cetin[6], Ugur Celik[1], Scott MacRae[1,2]

[1]Center for Visual Science. University of Rochester, Rochester, NY, USA
[2]Flaum Eye Institute, University of Rochester, Rochester, NY, USA
[3]The Institute of Optic., University of Rochester, Rochester, NY, USA
[4]Instituto de Óptica Daza de Valdés, CSIC, Madrid, Spain
[5]Department of Ophthalmology, Baylor College of Medicine, Houston, TX, USA
[6]Goergen Institute for Data Science and Artificial Intelligence, University of Rochester, Rochester, NY, USA

Purpose: With aging, the crystalline lens progressively loses its ability to accommodate (presbyopia) and maintain transparency (cataract). Lens replacement with an intraocular lens (IOL) is now the most frequently performed surgical procedure worldwide. However, current IOL selection routines rarely account for the preoperative crystalline lens geometry. Quantitative 3-D optical coherence tomography (OCT) enables full characterization of lens geometry, providing new opportunities to improve IOL positioning predictions, inspire accommodating IOL designs, and optimize lens sizing.

Methods: Three-dimensional lens geometry was obtained in vivo using OCT systems—both laboratory-developed and commercial instruments Fourier domain instruments—combined with refraction correction, geometrical distortion compensation, segmentation, and eigenlens basis representations. Extrapolation beyond the pupil was performed to recover the full lens shape in young subjects (to assess accommodation changes) and in patients before and after IOL implantation (n>100). Quantitative parameters included lens volume (VOL), equatorial diameter (DIA), lens thickness (LT), equatorial lens position (EPP), and eigenlens coefficients. Relationships between pre-operative geometry and post-operative IOL position (tilt, direction, and estimated lens position, ELP) were modeled using Gaussian Process Regression and Sequential Feature Selection (SFS).

Results: In young eyes, unaccommodated DIA averaged 8.9 mm and decreased by 0.6 mm/D with accommodation; LT increased and EPP decreased, while VOL remained constant. Eigenlens coefficients changed systematically with accommodation (Δa_2 = +1.92/D, Δa_1 = –0.6/D, Δa_5 = +0.4/D). In older subjects, VOL, LT, and DIA increased with age, with inter-subject variations of 100 mm³, 1.3 mm, and 1.2 mm at age 60. SFS revealed that post-operative IOL tilt depended mainly on pre-operative tilt, EPP, and IOL direction; ELP prediction error decreased by 50% when incorporating the top four ranked features, including ACD and a_1.

Conclusions: Quantitative 3-D OCT analysis of crystalline lens geometry provides predictive biomarkers for post-operative IOL alignment and position. Integrating these parameters into IOL selection routines could enhance surgical precision and guide next-generation accommodating IOL design.

Optical Coherence Elastography and Brillouin Spectroscopy of Crystalline Lens

Kirill Larin[1], Leana Rohman[2,3], Justin Schumacher[4], Christian Zevallos-Delgado[1], Manmohan Singh[1,] Bianca Maceo Heilman[2,3], Colette Shea[2,3], Marco Ruggeri[2,3], Arthur Ho[5], Salavat Aglyamov[1], Jean-Marie Parel[2,3], Giuliano Scarcelli[4], Fabrice Manns[2,3]

[1]Department of Biomedical Engineering, University of Houston, Houston, TX, USA
[2]Ophthalmic Biophysics Center, Bascom Palmer Eye Institute, University of Miami Miller School of Medicine, Miami, FL, USA
[3]Department of Biomedical Engineering, University of Miami College of Engineering, Coral Gables, FL, USA
[4]Department of Bioengineering, University of Maryland, College Park, MD, USA
[5]Brien Holden Vision Institute Ltd, Sydney, NSW, Australia
[6]Department of Mechanical Engineering, University of Houston, Houston, TX, USA

Purpose: Ex vivo measurements have demonstrated that the crystalline lens exhibits a gradient in mechanical stiffness that varies with both age and depth—young lenses possess a softer nucleus relative to the cortex, while older lenses become uniformly stiffer. Modeling studies indicate that this evolving gradient may drive overall lens hardening and presbyopia onset. The present study aimed to characterize the in vivo relationship between global lens stiffening and depth-resolved mechanical property distributions using a dual Brillouin–Optical Coherence Elastography (OCE) imaging platform.

Methods: Right eyes from 22 subjects aged 21–69 years were imaged using a custom-built multimodal Brillouin–OCE system under an IRB-approved protocol. The Brillouin module employed a 780 nm diode laser and a cross-axis, dual-stage VIPA spectrometer to obtain spatially resolved stiffness profiles within 12 s. The OCE module consisted of a phase-sensitive spectral-domain OCT at 842 nm combined with a 3.5 MHz acoustic radiation force (ARF) transducer targeting the anterior lens surface. After three Brillouin acquisitions, topical anesthesia was applied and the ARF probe—coupled with ultrasound gel—was positioned for OCE measurement. Brillouin data were analyzed to extract the central plateau and total profile thickness. OCE data were processed via a custom MATLAB algorithm to compute wave propagation speed and estimate Young's modulus.

Results: Aging was associated with a marked increase in both the overall and plateau thickness of Brillouin shift profiles. The nearly constant difference between total and plateau thickness across ages indicates that the stiff central zone contributes most to the thickening trend. Correspondingly, ARF-OCE–derived Young's modulus values increased substantially with age—from approximately 9.1–16.3 kPa in subjects aged 23–30 years to 40.7–76.2 kPa in those aged 47–69 years.

Conclusions: These findings demonstrate that age-related lens stiffening is closely linked to the formation and expansion of a high-modulus central plateau, likely reflecting cumulative compaction of lens fiber cells over time. This structural remodeling may underlie the biomechanical basis of presbyopia.

Acknowledgments: *National Eye Institute R01EY030063, P30EY14801, P30EY07551 (Center Core Grant); Florida Lions Eye Bank and Beauty of Sight Foundation; the Henri and Flore Lesieur Foundation (JMP); Drs. Harry W. Flynn, Jr*

Development of an Accommodating Intraocular Lens that Mimics the Young, Human Crystalline Lens

Liane Clamen[1], Daniel Stanley[1], Brett Thomes[1], Susana Marcos[2]

[1]Adaptilens Inc, Boston, MA, USA
[2]Center for Visual Science, University of Rochester, Rochester, NY, USA and Institute of Optics, Madrid, Spain

Purpose: No Intraocular Lens currently on the market can provide a full range of excellent quality vision, from near to far, with no negative side effects (halos, glare). The soft, flexible Adaptilens Accommodating IOL (A-IOL) was designed to provide excellent quality vision over the full visual range by mimicking the young human crystalline lens (HCL). The proprietary filling material and innovative lens design were created to mimic mechanical and optical properties of the HCL to respond to the eye's natural signal to change shape and provide high quality vision in response to visual stimuli at various distances without negative side effects. The Adaptilens A-IOL consists of a thin, flexible shell that is filled with a proprietary polymer.

Methods: Published studies on accommodation and the young HCL were utilized to define criteria for both the A-IOL shell material, filling material, and lens design. The proprietary filling material was formulated to match the optical and mechanical properties of the HCL. Capsular bag and ciliary body parameters from published literature were then utilized for use in Finite Element Analysis (FEA) to determine the critical factors for the lens design/material including component requirements to maximize accommodation. These results were used to manufacture A-IOLs which were then subjected to simulated accommodation bench tests.

Results: Accommodation simulation data demonstrated a consistent accommodation amplitude (> 2.5 D) with a force less than 80 mN. MTF = 0.79 at 50 lp/mm and MTF = 0.60 at 100 lp/mm across the entire accommodation range (see figure attached).

Conclusions: These data demonstrate that the shell material, filling material, and lens design together create an A-IOL that mimics the young HCL. Importantly, the Adaptilens A-IOL produces high quality vision over the full visual range as the lens changes shape from near to distant stimuli.

Finite element models of accommodation for estimation of crystalline lens material properties

Andres de la Hoz[1], Eduardo Martinez-Enriquez[1], Susana Marcos[2]

[1]*Instituto de Óptica Daza de Valdés, CSIC, Madrid, Spain*
[2]*Center for Visual Science. The Institute of Optics. Flaum Eye Institute, University of Rochester, Rochester, NY, USA*

Purpose: The mechanical properties of the crystalline lens are related to its optical function of accommodation and believed to be one of the factors that causes presbyopia. In this work we show the use of finite element models of accommodating crystalline lenses to estimate the mechanical properties of accommodating subjecting, by matching simulation results to experimentally retrieved surfaces obtained via OCT. In addition, we expand this method to a broader estimation using eigenlenses, a model for lens shape representation.

Methods: A finite element model of the accommodating crystalline lens was developed using ANSYS. Full lens shape data (from OCT and eigenlens representation) of 11 subjects (ages 22-30) was used for estimation of material properties for subjects; the lens shape at -4.5D demand was used for the initial geometry and the shape at 0D was used for comparison between experimental and simulated data. For the method expansion, an eigenlens database was used to generate 180 realistic lenses (ages 10-70, mean=32.4 yr) at maximum accommodative demand. These lenses were evaluated for 56 combinations of material properties of nucleus and cortex, and their shapes were represented in eigenlens form.

Results: For patient data, the estimated shear moduli of the nucleus and cortex were 1.62 +- 1.32 and 8.18 +- 5.63 kPa and correlated with age. Eigenlens representation of lens in initial and final states shows strong correlation with material properties.

Conclusions: Finite element models can be a useful tool for estimating the crystalline lens' mechanical properties. The use of eigenlenses model for describing lens geometry can result in a more robust and efficient estimation method.

Optical coherence tomography to study the gradient index of refraction of the human crystalline lens

Alberto de Castro[1], Eduardo Martínez-Enríquez[1], Susana Marcos[1,2]

[1]Instituto de Óptica Daza de Valdés, CSIC, Madrid, Spain
[2]Center for Visual Science. The Institute of Optics. Flaum Eye Institute, University of Rochester, Rochester, NY, USA

Purpose: The crystalline lens undergoes morphological and functional changes with age. The effects of the natural age-related process are well known, but to study the changes in its optical properties in vivo is still challenging due to its shell-like structure that originates a gradient in its physical properties, in particular its refractive index. The crystalline lens gradient index of refraction (GRIN), that peaks near the center and decrease toward the surfaces, can be reconstructed using Optical Coherence Tomography and its study can help to clarify the changes that take place at the crystalline lens with myopia, presbyopia and cataracts.

Methods: Optical coherence Tomography (OCT) is an interferometric imaging technique that is commonly used to image the human eye using near-infrared wavelengths. The image is formed with the time of flight and results in a measurement of the optical path between both surfaces travelled by the rays. The parameters of the GRIN distribution that best fit the experimental data can be obtained with a global optimization algorithm. Ray tracing was used to study the optics of the isolated crystalline lens and that of a full-eye model.

Results: The method allowed studying the GRIN distribution in human donor crystalline lenses of different ages. The optical properties of the crystalline lens were studied with the resulting distribution and with an equivalent refractive index, i.e. the homogeneous index of a lens with the same geometry and dioptric power, a common approximation when modelling the crystalline lens. The use of an equivalent homogeneous refractive index results in an overestimation of the spherical aberration. Simulations with full-eye models show that the use of an age-dependent GRIN model attenuates the differences.

Conclusions: OCT can be used to study the shell-like structure of the crystalline lens and to improve the geometrical and optical models the crystalline lens.

Eigenlens geometrical representation of the crystalline Lens

Eduardo Martínez-Enríquez[1], Alberto de Castro[1], Susana Marcos[1,2]

[1]Instituto de Óptica Daza de Valdés, CSIC, Madrid, Spain
[2]Center for Visual Science. The Institute of Optics. Flaum Eye Institute, University of Rochester, Rochester, NY, USA

Purpose: Knowing the full three-dimensional (3D) geometry of the crystalline lens is essential for studying age-related lens growth, accommodation mechanisms, and myopia development, as well as for improving intraocular lens (IOL) calculations in cataract surgery. Optical Coherence Tomography (OCT) is widely used to capture 3D images of the eye because it offers a good balance between speed and resolution and is readily available in clinical settings. However, OCT only provides access to the central portion of the lens that is visible through the pupil. We propose the eigenlenses representation, which enables accurate reconstruction of the full lens shape in vivo from OCT images, opening the door to a wide range of applications.

Methods: The eigenlenses representation was derived from OCT images of 133 donor lenses (ages 0–71 years), where the full crystalline lens shape, including the periphery, was visible after iris removal. Eigenlenses can represent the shape of any lens with high accuracy using only six numbers. Additionally, we designed and validated an estimation method that reconstructs the full lens shape in vivo from the partial region visible in OCT, using the eigenlenses representation.

Results: For the first time with optical imaging techniques, changes in the full crystalline lens shape (including equatorial diameter and volume) were analyzed as a function of age (n=475), myopia, and accommodation (n=14). IOL position estimation during cataract surgery was improved with the eigenlenses method compared to state-of-the-art techniques (n=368). Moreover, the eigenlenses method was used to generate realistic age-matched crystalline lens shapes (n=1000), which can serve as inputs for ray-tracing and biomechanical simulation programs to study lens optics and accommodation.

Conclusions: The eigenlenses method enables accurate estimation of the full crystalline lens shape from OCT images, with broad applications in the study of myopia, presbyopia, cataracts, and accommodation.

The Kinoshita Lecture

Thursday, December 11th
5:30 p.m.

John I Clark, PhD

University of Washington
Seattle, WA, USA


Friday, December 12th

Friday, December 12th

Session 14: Molecular Aging in Lens and Non-Lens Tissues

Moderators: *Kevin Schey & Christina Karakosta*

Talks are in the format of 15 minutes presentation with 3 minutes discussion.

Time	Speaker	Title
8:30 am	**Christina Karakosta**	Comparative Proteomic Analysis of Aqueous Humour, Anterior Lens Capsules, and Crystalline Lenses in Different Human Cataract Subtypes versus Healthy Controls
8:48 am	**Sarah Zelle**	Analysis of adult Nrf2 and αB-crystallin mutant zebrafish lenses exposed to oxidative stress reveals shifts in the lens proteome
9:06 am	**Preston Hazen**	N101D Deamidation and Cholesterol Content Differentially Impact the Membrane Binding of αA-Crystallin to Model Human Lens Lipid Membranes
9:24 am	**Kevin Schey**	Molecular Aging in the Lens: What are We Missing?
9:42 am	**Lee Goldstein**	Molecular Aging and Diabetic Protein Glycation Detected and Tracked In Vivo by Quasi-Elastic Light Scattering Spectroscopy Scanning of the Lens

Comparative Proteomic Analysis of Aqueous Humour, Anterior Lens Capsules, and Crystalline Lenses in Different Human Cataract Subtypes versus Healthy Controls

Christina Karakosta[1], Marilita M. Moschos[1], Anastasios Bisoukis[2], Nantieznta Kyriakidou[3], Konstantinos Moschou[3], Martina Samiotaki[4]

[1]1st University Eye Clinic, National and Kapodistrian University of Athens, "G. Gennimatas" General Hospital of Athens, Greece
[2]Bristol Eye Hospital, University Hospitals Bristol NHS Foundation Trust, Bristol, UK
[3]Day Unit Clinic DIATHLASIS, Thessaloniki, Greece
[4]Biomedical Sciences Research Center "Alexander Fleming", Vari, Greece

Purpose: To investigate the pathophysiology of cataract by analyzing proteomic signaling pathways in three samples types obtained from 4 different lens groups: age-related cataract (ARC), diabetic cataract (DC), post-vitrectomy cataract (PVC) and clear control lenses.

Methods: Three sample types - the aqueous humour, the anterior capsule and the phaco cassette content-were collected during cataract surgery from 39 participants, specifically from 12 participants with ARC, 11 with DC, 7 with PVC and 9 controls who underwent clear lens extraction for refractive reasons. The samples were prepared based on Sp3 protocol. The recognition and quantification of proteins was performed with liquid chromatography online with tandem mass spectrometry. Data analysis was performed using the DIA-NN software for protein identification/quantification. Perseus software was used for statistical analysis.

Results: In total, 1986 proteins were identified in the aqueous humour, 2804 in the anterior capsule, and 3337 in the phaco cassette samples. Proteins involved in actin and microtubule cytoskeleton organization, including ACTN4, DCTN1, MYO18A, TUBA1C, TBCB and TUBB4A, were downregulated in all 3 cataract groups, compared to controls. Proteins involved in glycolipid metabolic process, including GAL3ST1, GAL3ST4, and GLA, were upregulated in ARC, but downregulated in DC and PVC compared to controls. Proteins involved in the non-canonical Wnt receptor signaling pathway, including FRZB, SFRP1, SFRP2, SFRP5, WNT5A, and WNT7A, were upregulated in ARC, compared to DC, PVC, and controls.

Conclusions: Comprehensive proteomic profiles were generated using DIA proteomics, by comparing ARC, DC, PVC and controls. This is the first study to use phaco cassette contents to investigate cataract formation in comparison to controls. Our findings significantly advance the current understanding of human cataract pathophysiology and provide novel insights into the molecular mechanisms underlying cataract formation.

Analysis of adult Nrf2 and αB-crystallin mutant zebrafish lenses exposed to oxidative stress reveals shifts in the lens proteome

Sarah R. Zelle[1], Hassane S. Mchaourab[2], Kevin L. Schey[3]

[1]*Chemical and Physical Biology Program, Vanderbilt University, Nashville, TN, USA*
[2]*Department of Molecular Physiology and Biophysics, Vanderbilt University, Nashville, TN, USA*
[3]*Department of Biochemistry, Vanderbilt University, Nashville, TN, USA*

Purpose: Oxidative stress is thought to be important in age-related cataract (ARC) development, but how oxidative damage in the lens leads to cataract formation is not entirely clear. Previous results suggested that the oxidative stress response pathway can alter the proteostatic balance within the lens. We hypothesized that ARCs are a result of proteomic changes that occur due to an age-related compromised oxidative stress response pathway that synergistically interacts with the lens proteostatic state. To test this hypothesis, mass spectrometry proteomic analyses were conducted to better understand the oxidative and proteostatic stress responses *in vivo*.

Methods: Zebrafish models were employed as a mimic for reduced oxidative stress protection found in aged human lenses (Nrf2 mutant) and perturbed lens proteostatic state (αB-crystallin knockout). Oxidative stress was induced via hydrogen peroxide injections into the aqueous humor and resulting cataracts were characterized and quantified using grayscale values of lens images. Additionally, proteomic alterations in the cortex region of these lenses were studied using data-independent acquisition Parallel Accumulation–SErial Fragmentation mass spectrometry.

Results: Hydrogen peroxide treatment resulted in the formation of cortical cataracts. Significance testing revealed that the opacities in the hydrogen peroxide treated lenses were higher than control, PBS injected, lenses and only wild type treated lenses had a significant decrease in opacity over time. Proteomic results revealed that in all genotypes, hydrogen peroxide treated lenses had reduced ribosome and translation associated proteins and increased levels of aqueous humor-derived proteins. Furthermore, changes to capsule proteins were observed in the Nrf2 mutant lenses.

Conclusions: Induction of oxidative stress and ARC formation causes a shift in the lens and potentially aqueous humor proteomes through independent mechanisms in the Nrf2 mutant and αB-crystallin knockout models. This study further characterized the proteostatic and oxidative stress responses in the lens using cutting edge mass spectrometry proteomics techniques.

N101D Deamidation and Cholesterol Content Differentially Impact the Membrane Binding of αA-Crystallin to Model Human Lens Lipid Membranes

Preston Hazen[1], Nawal K. Khadka[2], and Laxman Mainali[1,2]

1Biomolecular Sciences Graduate Program, Boise State University, Boise, ID, USA
[2]Department of Physics, Boise State University, Boise, ID, USA

Purpose: αA-crystallin (αAc) becomes increasingly deamidated and membrane-bound with cataract formation. Moreover, the lens lipids and cholesterol (Chol) composition have been found to significantly change with both age and cataracts. Despite this, how membrane composition and deamidation affect the membrane interactions of αAc remains unclear. The aim of this study is to alleviate this gap in knowledge by analyzing the role of N101D deamidation and membrane composition on the membrane interactions of αAc.

Methods: We used electron paramagnetic resonance (EPR) to analyze wild-type (WT) and N101D-αAc membrane binding (% membrane surface occupied and binding affinity) and its effects on membrane physical properties (mobility, order, and hydrophobicity) to 60-year-old model human lens lipid (MHLL) membranes containing either 0, 16.7, 37.5, or 60 mol% Chol and 2 mol% Chol analog spin label.

Results: WT- and N101D-αAc bind to Chol/MHLL membranes with 0 and 16.7 mol% Chol in a saturable manner with no significant difference in binding. However, at 37.5 mol% Chol, WT-αAc has significantly higher binding than N101D αAc, and neither has any binding at 60 mol% Chol. At all Chol contents where there was αAc binding, N101D deamidation significantly reduced the binding affinity of αAc to the Chol/MHLL membranes. Lastly, WT and N101D-αAc membrane binding also showed to decrease membrane mobility, increase order, and increase hydrophobicity near the headgroup region. However, each increase in Chol content diminishes αAc membrane binding and these consequential changes to membrane properties.

Conclusions: This data ultimately indicates that Chol content modulates the binding of WT- and N101D-αAc with membranes differentially, with lower Chol content completely inhibiting the binding of N101D-αAc. Moreover, these results indicate that the continual increase in the lens Chol content found with healthy aging is critical to preventing WT and deamidated αAc membrane binding and likely protecting against cataract formation.

.

Molecular Aging in the Lens: What are We Missing?

Kevin L. Schey[1], Zhen Wang[1], Jarod Fincher[1], Sergei Chetyrkin[1]

[1]*Department of Biochemistry, Vanderbilt University, Nashville, TN, USA*

Purpose: Proteins and lipids play essential cellular roles in signaling, differentiation and as structural components of the cell. Proteins and lipids in lens fiber cells reside in place for decades and thus are subject to age-related modification. The current report examines protein and lipid changes with age and cataract with an emphasis on detecting novel molecular species and their spatial localization.

Methods: Both imaging mass spectrometry (IMS) and spatially-resolved proteomics/lipidomics analysis were used to spatially map protein and lipid composition in transparent and cataractous human lenses (aged 19-70y). Frozen human lenses were cryosectioned to 12 micron thickness, coated with MALDI matrix proteins and lipids imaged by MALDI IMS. Additionally, lenses were dissected into cortical and nuclear regions followed by protein digestion for proteomics analysis or by MMC extraction of lipids and LC-MS/MS based lipidomics analysis.

Results: Consistent with previous publications, proteomic analysis revealed a plethora of age-related modifications and cataract-specific modifications. Some modifications were only detected in non-standard negative ion mode conditions. MALDI-IMS analysis revealed regions of specific lipid localization corresponding to fiber cell age. Glycerophospholipids were predominant in the lens cortex and sphingomyelin lipids in the lens nucleus. Interestingly, large gangliosides (GM1, GM3) were detected in cortical fiber cells, whereas smaller gangliosides (GA1 and GA2), glucosylceramides, and lactosylceramides, were detected in older nuclear fibers, suggesting an age-related degradation of lens gangliosides. Cataract-specific changes in the lens cortex include more ether linked lipids and lipids with odd chain fatty acids.

Conclusions: Spatial lipidomics suggests that, like proteins, lipid expression is highly spatially regulated and that lipid modification occurs with age in long-lived cells or extracellular deposits. Identification of novel molecular species suggests that additional modifications remain to be discovered.

Molecular Aging and Diabetic Protein Glycation Detected and Tracked In Vivo by Quasi-Elastic Light Scattering Spectroscopy Scanning of the Lens

Lee E. Goldstein[1a,b,c,d,2,3], Douglas S. Parsons[1a], Olga Minaeva[1a], Juliet A. Moncaster[1a]

[1]Departments of [a]Radiology [b]Psychiatry, [c]Neurology, [d]Pathology and Laboratory Medicine, Chobanian & Avedisian School of Medicine, Boston University, Boston, MA, USA
[2]Department of Biomedical Engineering, Boston University College of Engineering, Boston, MA, USA
[3]Boston University Alzheimer's Disease Research Center, Boston University, Boston, MA, USA

Purpose: New technology is needed for objective point-of-care assessment of individual variation in molecular aging and risk for diabetic complications. Enabling technology will allow clinicians to detect and track objective biomarker metrics linked to the biology driving molecular aging and diabetic end-organ damage. Development of such precison medicine tools are essential for maximizing health throughout life ("healthspan").

The lens is an ideal tissue to assess molecular events associated with aging and diabetes. Long-lived proteins are expressed during fetal life, do not undergo turnover, and accumulate post-translational modifications and structural changes throughout life. We developed a scanning laser ophthalmoscope with integrated quasi-elastic light scattering (QLS) spectrometer that enables noninvasive assessment of molecular changes in the lens. We hypothesized that alterations in QLS lens signals constitute an *in vivo* biomarker of molecular aging and diabetes will accelerate these changes. We evaluated QLS lens signals in living C57BL/6 mice as a function of age and diabetes mellitus (DM). We also conducted a cross-sectional clinical validation study in patients with type-1 DM (T1D).

Methods: We used quasi-elastic light scattering (QLS) to measure: (i) age-dependent lens changes in unanesthetized C57BL/6 mice (4–18 mos), (ii) glycemia-dependent lens changes in unanesthetized C57BL/6 mice (4–18 mos) treated with streptozotocin (STZ, T1D model), (iii) cross-sectional analysis of human subjects with T1D vs non-diabetic controls. Retinal damage was assessed in the mice using electroretinography and histopathology.

Results: QLS metrics of molecular aging can be acquired noninvasively in unanesthetized mice and human subjects with and without diabetes. Consistent with our hypotheses, QLS metrics were significantly altered in lenses of mice and humans with advancing age and with diabetes compared to non-diabetic controls.

Conclusions: Our findings demonstrate that QLS analysis of lens proteins provides a practical, clinically-relevant technique for noninvasive assessment of molecular aging and long-term hyperglycemic status *in vivo*.

Friday, December 12th

Session 15: No Lens is an Island

Moderators: *Steve Bassnett & Katelyn Swindle-Reilly*

Talks are in the format of 15 minutes presentation with 3 minutes discussion.

Time	Speaker	Title
10:30 am	**Katelyn Swindle-Reilly**	The Lens Is Not an Island: Strategies from the Vitreous to Support Lens Health
10:48 am	**Jodi DeDreu**	How the lens functions in regulating immune responses to maintain/restore homeostasis in the eye
11:06 am	**Marco Ruggeri**	Quantifying the Ciliary Muscle in Accommodation with Optical Coherence Tomography
11:24 am	**Juan Rodriguez**	Tweezing out zonular mechanics
11:42 am	**Steve Bassnett**	Developing an Anatomically Accurate Base Model of the Human Eye with Emphasis on the Zonular Apparatus

The Lens Is Not an Island: Strategies from the Vitreous to Support Lens Health

Katelyn E. Swindle-Reilly[1,2,3], Meghal Keskar[2], Heather L. Chandler[4], Matthew A. Reilly[1,3]

[1]Department of Biomedical Engineering, The Ohio State University, Columbus, OH, USA
[2]William G. Lowrie Department of Chemical and Biomolecular Engineering, The Ohio State University, Columbus, OH, USA
[3]Department of Ophthalmology and Visual Sciences, The Ohio State University, Columbus, OH, USA
[4]College of Optometry, The Ohio State University, Columbus, OH, USA

Purpose: While lens aging and cataractogenesis are often attributed to oxidative stress, emerging evidence suggests that the adjacent vitreous body plays a critical role in maintaining lens health. The vitreous humor has long been considered disposable, just like the lens in terms of cataract being a solved problem. This study presents an engineering perspective on the role of the vitreous humor, particularly its biomechanical and biochemical properties, in preserving ocular health and protecting against lens oxidative damage. We have investigated biomaterial-based strategies to restore vitreous functionality.

Methods: Antioxidant-loaded hydrogel systems were engineered to mimic the native vitreous environment including viscoelasticity. Antioxidants including ascorbic acid, glutathione, and ergothioneine were explored for encapsulation in vitreous substitutes to mitigate oxidative stress after vitrectomy. Material properties, release kinetics, and antioxidant activity have been characterized *in vitro* as well as in an *in vivo* pilot study.

Results: Nanoencapsulation of the antioxidants prolonged the duration of release from a hydrogel. Synergistic combinations of antioxidants appear to reduce oxidative stress better than individual antioxidants. Treated eyes exhibited reduced oxidative stress markers in both the vitreous and lens after 1 month *in vivo*. No adverse tissue responses were observed, confirming tolerability of high doses of antioxidants in the eye.

Conclusions: These findings and emerging evidence support a more holistic view of lens biology, where the vitreous likely directly influences lens health. Restoring antioxidant balance in the vitreous may offer a novel therapeutic avenue for delaying or preventing cataract formation. This work highlights the need to consider the lens within its broader ocular microenvironment and calls for lens researchers to work together with other eye researchers to emphasize the importance of both the lens and vitreous in ocular health.

How the lens functions in regulating immune responses to maintain/restore homeostasis in the eye

JodiRae DeDreu[1], Phuong M Le[1], Mary J. Mattapallil[2], Rachel R Caspi[2], Mary Ann Stepp[3], and A. Sue Menko[1]

[1]Department of Pathology and Genomic Medicine, Sidney Kimmel Medical College, Thomas Jefferson University, Philadelphia, PA, USA
[2]Laboratory of Immunology, National Eye Institute, National Institutes of Health, Bethesda, MD, USA
[3]Department of Anatomy and Cell Biology, George Washington University School of Medicine and Health Sciences, Washington, DC, USA

Purpose: We investigated a novel role for the lens as a site for recruitment of immune cells that restore and maintain homeostasis in the eye in response to wounding and inflammation.

Methods: Multiplex immunofluorescence labeling of mouse eyes was performed in the context of corneal wounding, corneal erosions and Experimental Autoimmune Uveitis (EAU). Images were acquired with a Zeiss 800 confocal microscope and analyzed using ZEN or Imaris software.

Results: We discovered that immune cells are recruited to the surface of the lens capsule in response to corneal wounding, corneal erosion formation and EAU inflammation. The nature of the ocular insult influences the localization and phenotype of the recruited Lens Capsule Associated Immune Cells (LCA-ICs), as well as the duration of their association with the lens surface. In corneal wounding, LCA-ICs are recruited to the anterior surface of the lens within 24hrs post-injury and absent by day 3 when the wound is closed. With development of corneal erosions, LCA-ICs are again recruited whose phenotypes include neutrophils/PMN-MDSCs and M2 macrophages, which parallel the immune cell phenotypes populating the corneal stroma directly under the erosion site. In EAU, LCA-ICs are primarily M2 macrophages with immunosuppressive properties and Tregs. LCA-ICs persist in association with all regions of the lens capsule throughout resolution of EAU inflammation. LCA-ICs travel to the lens from the ciliary body along the ciliary zonule fibrils. In EAU their long-term presence causes capsule stiffening that can lead to cataractogenesis.

Conclusions: Through recruitment of immune cells to the lens capsule surface, the lens plays a vital role in regulating inflammatory responses in the eye and maintaining homeostasis.

Quantifying the Ciliary Muscle in Accommodation with Optical Coherence Tomography

M. Ruggeri[1,2], Y.-C. Chang[1], L. Rohman[1, 2], C.G. Iulen[3], H. Durkee[1], F. Cabot[1,4], B. Maceo Heilman[1], G. Monterano Mesquita[1,2], A. Ho[1,5,6], F. Manns[1, 2]

[1]Bascom Palmer Eye Institute, University of Miami Miller School of Medicine, Ophthalmic Biophysics Center, Miami, FL, USA
[2]University of Miami College of Engineering, Biomedical Engineering, Coral Gables, FL, USA
[3]University of Zaragoza, Aragón Institute of Engineering Research (i3A), Zaragoza, Spain
[4]Bascom Palmer Eye Institute, University of Miami Miller School of Medicine, Anne Bates Leach Eye Hospital, Miami, FL, USA
[5]Brien Holden Vision Institute, Sydney, Australia
[6]University of New South Wales, School of Optometry & Vision Science, Sydney, Australia

Purpose: The ciliary muscle plays a fundamental role in accommodation and presbyopia, but in vivo assessment remains challenging due to its location and dynamic function. We present methods based on Optical Coherence Tomography (OCT) to quantify ciliary muscle displacement and morphology during accommodation and aging, and report findings from studies testing the effect of presbyopia drops and accommodating intraocular lenses (IOLs) on accommodative structures in presbyopic patients.

Methods: Custom spectral-domain and swept-source OCT systems were developed to simultaneously image the ciliary muscle transsclerally and the crystalline lens at high speed and resolution. Dynamic imaging protocols recorded OCT image sequences during controlled accommodative stimuli. Automated deep-learning segmentation delineated the anterior and posterior ciliary muscle boundaries, and landmark-based registration enabled analysis of apex and centroid displacements and shape descriptors. Quantitative metrics were compared across young and presbyopic subjects, in eyes implanted with accommodating IOLs, and before and after instillation of presbyopia drops (pilocarpine).

Results: OCT visualized the ciliary muscle from the scleral spur to its posterior border. In young subjects, accommodation produced significant apex and centroid displacements, centripetal motion, and thickening, whereas presbyopic subjects showed reduced mobility. Dynamic imaging confirmed temporal coupling between ciliary muscle motion and lens shape changes, with muscle displacement often preceding lens thickening. In the pilocarpine study, pupil diameter decreased significantly versus control, but no significant changes in lens thickness or ciliary muscle response were observed. In eyes with accommodating IOLs, neither the IOL nor the ciliary muscle demonstrated measurable accommodative response.

Conclusions: Quantitative OCT imaging provides objective assessment of ciliary muscle morphology and function across accommodation and aging. This approach offers insights into presbyopia mechanisms and enables direct evaluation of how pharmacological agents and accommodating IOLs affect accommodative structures.

Tweezing out zonular mechanics

Juan Rodriguez[1]

[1]*University of Health Sciences and Pharmacy in St. Louis, St. Louis, MO, USA*

Purpose: Zonular fibers are elastic structures that play a critical role in maintaining lens position and transmitting forces from the ciliary muscles to enable accommodation. They are remarkable for their capacity to withstand continuous mechanical loading over a lifetime of eye movement and rapid accommodative shifts. Here we present results obtained with an apparatus recently developed in our laboratory for investigating their mechanical properties and resilience, including responses to static and dynamic strains, enzymatic degradation, and chemical fixation.

Methods: Bovine and human eyes were dissected to expose the lens, zonular fibers, ciliary body and a surrounding ring of sclera is pinned to the base of a Petri dish positioned on the stage of a dissecting fluorescence microscope. The mechanical properties of a single zonular fiber were assessed using a conceptually straightforward approach, schematically illustrated two mechanically controlled pairs of forceps, operated under the dissecting microscope, to grasp the ends of a fiber within a dissected eye. The eye is then lowered to isolate the fiber; the dish with the dissected eye is removed and replaced with an experimental dish containing the appropriate testing solution. A hook connected to a force transducer is positioned just beneath the fiber midpoint and gradually raised to apply tension. The viscoelastic behavior of the fiber was quantified by combining the transducer's force measurements with geometric parameters such as fiber length and diameter, which were determined from microscope images. These values were used alongside the upward displacement of the probe. Tests were conducted in solutions containing α-amylase, trypsin, hyaluronidase, paraformaldehyde, ethanol, and cyclohexane. Additionally, fibers were deposited onto glass substrates using the same apparatus and subsequently examined by atomic force microscopy (AFM).

Results: All fibers exhibited stress relaxation, which became more pronounced at higher strain levels. Fiber stiffness was markedly reduced following exposure to trypsin and the carbohydrate-digesting enzyme α-amylase, but not to hyaluronidase, despite prior reports of hyaluronidase co-localization with zonular fibers. In contrast, treatment with ethanol and cyclohexane significantly increased fiber stiffness while reducing fiber diameter. Paraformaldehyde exposure produced no measurable effect. Atomic force microscopy (AFM) imaging further suggested that the fibers are coated with an amorphous surface layer of as-yet undetermined composition.

Conclusions: The relaxation behavior of zonular fibers is consistent with that generally observed in hydrated tissues, suggesting that hydration contributes significantly to their mechanical performance. This interpretation is supported by the effects of ethanol and cyclohexane, both of which promote dehydration. The pronounced reduction in stiffness following α-amylase treatment further implicates carbohydrates as key structural and/or mechanical components of these fibers. Given the well-documented protective effects of carbohydrates against chemical attack, their presence may also account for the fibers' insensitivity to paraformaldehyde exposure and, more broadly, their remarkable durability and longevity throughout a lifetime of ocular function.

Developing an Anatomically Accurate Base Model of the Human Eye with Emphasis on the Zonular Apparatus

Steven Bassnett[1], Pooja Rathaur[1]

[1]Department of Ophthalmology & Visual Sciences, Washington University School of Medicine, St. Louis, MO, USA

Purpose: The structural organization of the human eye has been studied since Zinn's *Descriptio Anatomica Oculi Humani* (1755). Modern imaging modalities such as OCT, ultrasound, and scanning laser ophthalmoscopy allow visualization of the living eye, whereas conventional histology and electron microscopy are applied ex vivo. Each technique has advantages and limitations, and some structures remain inaccessible because of their location or their interaction with imaging energy. Furthermore, the very different spatial scales of these methods (e.g., electron microscopy vs. ocular ultrasound) makes integration of the resulting data sets challenging. To address this, we used Blender, an open source, mesh-based 3D modeling platform, to construct an anatomically accurate base model of the human eye.

Methods: A 70-year-old human eye served as the reference model. Biometric values were compiled from published data sets, and approximately 40 human globes were examined directly. Tissue surfaces were visualized by confocal microscopy after labeling with Alexa488-phalloidin and DAPI, and the zonular apparatus was stained with fluorescent lectin. Additional fixed globes were treated with osmium or Lugol's Iodine and imaged by X-ray microscopy. The base model was constructed in Blender 4.0.

Results: We compiled a comprehensive biometric data set for the 70-year-old human eye and used it to create a mesh model in Blender, incorporating our recent findings on the structural organization of the zonular apparatus.

Conclusions: Because the model is derived from referenceable biometric and imaging data, it provides a robust foundation for future finite element simulations of accommodation. Animated versions may also offer insights into structural changes that occur during ocular development and aging.

PRESENTING AUTHOR INDEX