
ISER
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ISER / BRIGHTFOCUS GLAUCOMA SYMPOSIUM:
CONCEPTS AND
BREAKTHROUGHS
IN GLAUCOMA
ABSTRACT BOOK
ATLANTA, GEORGIA, USA
OCTOBER 8 - 11, 2025

# Glaucoma Symposium Schedule at a Glance

| | WEDNESDAY, OCTOBER 8, 2025 | THURSDAY, OCTOBER 9, 2025 | FRIDAY, OCTOBER 10, 2025 | SATURDAY, OCTOBER 11, 2025 |
|---|---|---|---|---|
| 6:30 AM - 8:00 AM | | 6:30 AM - 7:50 AM Breakfast | Networking Breakfast | Breakfast |
| 8:00 AM - 8:45 AM | | 7:50 AM - 8:45 AM Keynote Lecture 1 The Complex Genetics of Glaucoma: Biological Insights and clinical prediction | Keynote Lecture 2 The Ins and Outs of the Eye's Aqueous Humor | Keynote Lecture 3 Axonal Mitochondria and the Starry Night |
| 8:45 AM - 10:05 AM | | Platform Session 1 Genetics: Blame It on Your Parents! | Young Investigators Session Platform Session 4 The Next Gen-EYE-ration | 8:45 AM - 10:30 AM Platform Session VIII Glial Cells: The Good, the Bad and the Ugly? |
| 10:05 AM - 10:45 AM | | Break | Break | 10:30 AM-11:00 AM Break |
| 10:45 AM - 12:30 PM | | Platform Session 2 You're Stressing Me Out! | Platform Session 5 Regeneration & Neuroprotection: I Will Survive! | 11.00 AM - 12.20 PM Platform Session IX EYE am Under Pressure :-( |
| 12:30 PM - 1:45 PM | | Lunch | Lunch | |
| 1:45 PM - 3:30 PM | | Platform Session 3 Get Real! - Translation | Platform Session 6 What You Need to Know About Glaucoma | |
| 3:30 PM - 4:00 PM | | Break | Break | |
| 4:00 PM - 5:15 PM | | Poster Session Hanging out with Brilliant Ideas | Platform Session 7 Go with the Flow | |
| 5:15 PM - 6:30 PM | Kick-Off Reception | | Break | |
| 6:30 PM - 8:00 PM | Dinner | Dinner | Gala Dinner | |

# PLATFORM SESSION 1
# GENETICS: BLAME IT ON YOUR PARENTS!

## 8:45 AM - 10:25 AM

## EMORY AMPHITHEATER

## EFFECT OF AGING ON THE MOUSE SCHLEMM'S CANAL AND TRABECULAR MESHWORK AT SINGLE-CELL RESOLUTION

**Revathi Balasubramanian[1], Guorong Li[2], Nicholas Tolman[1], Vaibhav Jain[2], Krishnakumar Kizhatil[3], W. Daniel Stamer[2], Simon John[1]**

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Aging and ocular hypertension (OHT) are the primary risk factors for glaucoma development. Increased stiffness of trabecular meshwork (TM) and altered mechanotransduction across the Schlemm's canal (SC) positively associates with elevated outflow resistance and OHT. Understanding the molecular mechanisms by which aging affects SC and TM cells, key cell population responsible for regulating intraocular pressure (IOP), is crucial. This study aims to investigate the impact of aging on the transcriptome of SC and TM cell subtypes, with the goal of elucidating the unique functions of SC and TM cells prone to outflow dysfunction and in the pathogenesis of OHT and glaucoma. We isolated single cells and single nuclei from limbal tissues of three age groups (young-3mo, middle-12mo, and old-24mo) of C57BL/6J mice for droplet-based RNA sequencing. A total of six cohorts of single cell and single nucleus samples were independently collected at two sites - Duke Genome Core facility and Columbia University Genome Center and integrated for analyses. We integrated the datasets, profiled ~100,000 transcriptomes) and identified various anterior segment cell types including the SC and the TM. A gene ontology pathway analysis of cells highly expressing *Myoc* (*Myoc*-hi TM1 cells) showed changes in extracellular matrix (ECM) structural constituents - integrin binding and collagen binding indicating that changes to ECM remodeling and rigidity are occurring with age in TM1 cells. We noted enhancement in immune regulation - TAP binding, NK-cell receptor binding, and CD8 receptor binding in the old TM1 cells. In *Acta2*-hi *Lmx1b*-hi old TM3 cells we noticed similar changes in ECM components and immune regulation, as well as changes in calcium ion transport and junctional organization suggesting altered TM function. In old SC cells, we noticed changes in response to oxidative stress pathways and an increased requirement of immune cells. In conclusion, age related changes include inflammation and fibrotic response in TM cells with some differences between cell subtypes, and response to chemical stress with increased immune requirement in SC cells. Further studies are necessary to understand how inflammation influences SC and TM tissue fibrosis, stiffness, impaired mechanotransduction and disease development.

## GENETIC REGULATION DISCOVERY IN THE AQUEOUS OUTFLOW PATHWAYS, MACULA AND OPTIC NERVE HEAD AND ITS APPLICATION FOR UNCOVERING GLAUCOMA MECHANISMS

**Puja Mehta[1], Rinaldo Catta-Preta[1], Abbi Engel[2], Jason Turner-Maier[1], Sudeep Mehrotra[1], Iris Cheng[1], Janey L. Wiggs[1], Timothy Cherry[2], Kinga Bujakowska[1], Ayellet Segre[1]**

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Primary open-angle glaucoma (POAG), characterized by retinal ganglion cell death, is the leading cause of irreversible blindness worldwide, yet it has no cure. Genome-wide association studies (GWAS) have identified > 300 genomic loci associated with POAG, most of which lie in noncoding regions. However, genetic regulatory effects have not been detected in glaucoma-relevant eye tissues. Understanding the etiology of glaucoma may be important for developing effective neuroprotective treatments. We aimed to identify expression and splicing quantitative trait loci (eQTLs, sQTLs) and open chromatin regions in human trabecular meshwork (TM)/Schlemm's canal (SC), ciliary body (CB), macula, and optic nerve head (ONH), and use them to uncover novel ocular-dependent regulatory mechanisms and genes that underlie glaucoma associations. We collected ten anterior and posterior eye tissues from 100 postmortem non-diseased donor eyes, and performed RNA-sequencing on TM/SC, CB, macula, and ONH from 100 donors, whole genome sequencing of all donors' DNA, and ATAC-seq on the four tissues from 6 donors. Quality control yielded 88 TM/SC, 91 CB, 94 macula and 89 ONH high-quality RNA-seq samples and ATAC-seq from 3-6 donors each. We detected tissue-specific and tissue-shared gene expression, splicing events quantified with LeafCutter, and > 100k open chromatin regions across these tissues. eQTL and sQTL mapping was applied to common variants in cis ( ± 1Mb) of all genes expressed in each tissue, adjusting for sex, age, top genotype PCs and inferred covariates. Significant e/sQTLs (FDR < 0.05) were assessed for tissue-specificity against 49 GTEx tissues. Colocalization analysis of the ocular e/sQTLs with POAG and intraocular pressure (IOP) loci and fine-mapping using overlapping ATAC-seq peaks proposed putative causal genes and regulatory mechanisms for POAG in the outflow pathways, ONH and retina. We present a first comprehensive transcriptomic and gene regulation atlas of key glaucoma tissues, which will be made available on VisionGenomics.org.

Acknowledgements: NIH/NEI-R01EY031424, Lions VisionGift Investigator-Concept Study Grant.

## LONG INTERGENIC NONCODING RNAS AS MOLECULAR SENSORS IN RETINAL GANGLION CELL DEGENERATION

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Glaucoma causes progressive retinal ganglion cell (RGC) death due to optic nerve damage, typically driven by elevated intraocular pressure (IOP). While lowering IOP is the mainstay of treatment, it does not prevent vision loss in all patients, underscoring the need for effective neuroprotective strategies. RGCs are highly heterogeneous, and certain subtypes, such as intrinsically photosensitive RGCs (ipRGCs), are more resilient to injury than others. To understand the molecular underpinnings of this resilience, we performed transcriptomic profiling of injury-resilient and injury-susceptible RGC types and discovered a large number of long noncoding RNAs (lncRNAs) that are differentially expressed after axonal injury. Among these, a previously uncharacterized long intergenic noncoding RNA (lincRNA), which we term optic nerve injury induced lncRNA (Onil1), was identified as highly upregulated in injury-susceptible RGCs. Following optic nerve injury, this lncRNA is upregulated in RGCs as early as 24 hours post-injury, implicating it in the initial molecular events leading to RGC death. Silencing Onil1 in RGCs using AAV-delivered shRNAs led to dramatic increase in RGC survival after optic nerve crush in adult mice, implicating Onil1 in neuronal degeneration. Silencing Onil1 promoted RGC survival and visual function recovery in an inducible model of ocular hypertension (i.e., intracameral silicone oil injection). This work is the first to uncover and investigate novel roles of lincRNAs in glaucoma-associated neurodegeneration and proposes that a RNA transcript Onil1 functions as an injury-responsive “sensor RNA” that regulates early pro-death pathways, including ATF3 and p-cJUN expression. Findings from this study will advance understanding of lncRNA-mediated neurodegeneration and may pave the way for lncRNA-targeted gene therapies in glaucoma and other optic neuropathies.

## SINGLE CELL RNA SEQUENCING OF LAMINA CRIBROSA FIBROBLASTS IDENTIFIES A KEY ROLE FOR THE INTEGRATED STRESS RESPONSE IN THE PATHOPHYSIOLOGY OF HUMAN GLAUCOMA OPTIC NERVE CUPPING

**Colm O'Brien[1], Caoimhe Normile[1], Oisín Cappa[2], Vadim Zhernovkov[3], Jeffrey O'Callaghan[4], Bruce Moran[5], David Simpson[2], Eoghan Culligan[1], Sherri Feris[6], Stacy Curry[6], Abbot Clark[6], Mustapha Irnaten[1]**

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Glaucoma is a leading neurodegenerative disease that progressively damages retinal axons in the optic nerve head and optic nerve. The molecular mechanisms that underlie the fibrotic extracellular matrix (ECM) remodelling of the lamina cribrosa (LC) in the human glaucomatous optic nerve remain unresolved. Analysis of single cell RNA-sequencing of LC cells from normal and glaucoma donors revealed 4 distinct clusters with 1144 differentially expressed genes, while lineage tracing identified a mesenchymal/fibroblast cell origin. The glaucoma fibroblasts over-expressed multiple pro-fibrotic genes, master transcription factor (TF) regulons involved in cell fate decisions, and TF motifs including SRF and multiple Integrated Stress Response (ISR) genes including ATF4, ATF3 and CREB3L1. The glaucoma cells had enriched cell cycle proliferation pathways (E2F, G2M, MYC), with downregulation of the p53 pathway. Inhibition of the ISR reduced the expression of pro-fibrotic genes and the increased cell proliferation rate in glaucoma LC fibroblasts, thereby confirming the recently identified key role of the ISR in the pathogenesis of human glaucoma.

## EXPANDING GENETIC DIVERSITY ENHANCES DISCOVERY OF POAG RISK VARIANTS ACROSS POPULATIONS

**Lauren Cruz[1], Tyler Kinzy[2], Osahon Asowata[2], Ayobami Alimi[2], Andrea Waksmunski[1], Jacquelaine Bartlett[3], Bryan Gorman[4], Christopher Halladay[5], Wen-Chiu Wu[5], Scott Williams[2], Michael Hauser[6], Dana Crawford[1], Sudha Iyengar[3], Neal Peachey[7], Jessica N. Cooke Bailey[2]**

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It is well-established that primary open-angle glaucoma (POAG) differs across populations, whether defined by genetic ancestry, ethnicity, or race. We performed the most comprehensive genetic analysis from African American (AFR) and Hispanic (HIS) groups with well-defined POAG to date via the Million Veteran Program (MVP). We also relaxed the parameters of POAG definition to better understand the role of phenotype definition stringency in genetic studies of POAG.

POAG status was defined from electronic health records for two phenotype definitions: POAGEXP includes a clinician-validated list of ICD 9/10, glaucoma-relevant procedure, visit, and medication codes, excluding conflicting diagnoses (age: cases ≥ 30, controls ≥ 65); POAGPheCode required ≥ 2 POAG phecode 365.11 (age ≥ 18). POAGEXP classified 234,153 European American (EUR) (10,738 cases, 223,415 controls), 37,931 AFR (6,889 cases, 31,042 controls), and 15,712 HIS (1,270 cases, 14,442 controls); POAGPheCode classified 453,512 EUR (24,268 cases, 429,244 controls), 119,055 AFR (16,083 cases, 102,972 controls), and 50,994 HIS (3,096 cases, 47,898 controls). We performed (a) assessment of GWAS Catalog data for POAG cases and controls by ancestry, (b) genome-wide association analyses (SAIGE 1.3.0, covariates: age, sex, 10 Principal Components) of MVP-release4 data (imputed to TOPMed-imputed, the most comprehensive reference panel for non-majority-European samples), and (c) cross-ancestry meta-analysis (MR-MEGA).

Applying both phenotype definitions, we identified 5, 13, and 24 novel POAG loci in EUR, AFR, and cross-ancestry analyses, respectively, at genome-wide significance (GWS: $p < 5x10-8$; 35 independent loci;) and detected GWS at TMCO1, FMNL2, CDKN2B, and CDC42BPA for the first time in HIS. We increase the percentage of POAG cases in the GWAS catalog from 16.8% to 24.4% for AFR and from 1.8% to 3.9% for HIS.

Our study demonstrates the power of diverse, well-phenotyped cohorts in uncovering novel genetic loci for POAG, substantially increasing the representation of African and Hispanic populations in the global glaucoma genomics landscape.

# PLATFORM SESSION 2
# YOU'RE STRESSING ME OUT!

## 10:45 AM - 12:30 PM

## EMORY AMPHITHEATER

## FACTORS DRIVING LAMINA CRIBROSA PERFUSION AND OXYGENATION SENSITIVITY TO ELEVATED IOP

**Ian A. Sigal[1], Yuankai Lu[1], Susannah Waxman[1], Yi Hua[2], Xinyue Wang[1], Qi Tian[1], Joel Palko[3]**

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The mechanisms by which elevated intraocular pressure (IOP) contributes to glaucomatous neuropathy remain unclear. A longstanding theory posits that elevated IOP distorts the vasculature within or supplying the lamina cribrosa (LC) region, reducing LC blood perfusion and oxygenation. We employed a combination of experimental and computational methods to quantify LC blood flow and tissue oxygenation. Experimental measurements of blood flow rates were obtained at baseline (15 mmHg) and elevated IOP (30 mmHg) using super-resolution ultrasound. These measurements served as boundary conditions for 3D LC vasculature models of four healthy eyes reconstructed from histology. In vivo acute IOP-induced LC deformations were measured from healthy monkeys using imaging and tracking techniques, which were then used to distort the models, impacting blood flow and oxygenation.

In a series of experiments, we identified the main factors determining LC hemodynamics and oxygenation: capillary diameters, the extent of IOP-induced distortions, and the routes of blood perfusing the LC. We found that eyes perfused through the central retinal artery in addition to peripheral perfusion were significantly more resilient to elevated IOP than those perfused solely from the periphery. The LC perfusion and oxygenation were remarkably resilient to the compromised flow resulting from IOP-induced distortions, primarily due to the heavily interconnected nature of the capillary network, which provided multiple pathways for blood flow and ensured that neural tissues had various sources of oxygen. The LC perfusion network is closely integrated with that of the prelaminar and retrolaminar regions. However, even with mild increases in IOP, there were regional reductions in oxygenation that, if sustained over long periods, could trigger biological effects. Our results suggest that approximately 30% of monkeys lack direct inflow from the CRA to the LC, placing them at an elevated risk of hypoxia even at mildly elevated IOP.

## CHARACTERIZATION OF OPTIC NERVE HEAD VISCOELASTIC PROPERTIES USING PHYSIOLOGICAL OCULAR PULSE AND VIRTUAL FIELD METHOD

**Xiaofei Wang[1], Qi Li[1], Yuejiao Jin[2], Michael Girard[3]**

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**Purpose:** To develop a noninvasive method for quantifying the viscoelastic properties of optic nerve head (ONH) tissues by leveraging natural ocular pulse-induced tissue deformations and the virtual fields method (VFM).

**Methods:** A VFM framework was implemented within the open-source finite element (FE) software FEBio. A viscoelastic constitutive model under steady-state conditions was used for the lamina cribrosa (LC) of the ONH. The viscoelastic model was defined by elastic modulus (E), viscoelastic coefficient (γ), and relaxation time (τ). Validation of the VFM method employed FE models of increasing complexity: single-element, 3D LC geometry and a whole-eye model, using known material parameters of the LC (E = 0.3 MPa; γ = 8; τ = 1 s). A multi-frequency approach (simulating heart rates of 60, 90, and 120 beats per minute) assessed strain-rate dependency effects. Clinical feasibility was tested using pulsatile displacements measured in vivo via low-coherence tissue interferometry (LCTI) from a healthy subject, combined with an estimated ocular pulse amplitude (3 mmHg).

**Results:** VFM accurately recovered input parameters in single-element and 3D LC models. Multi-frequency analysis showed minimal effects on the predictions. Whole-eye FE simulations yielded comparable results (E: 0.28 MPa, γ: 8.4, τ: 1.00 s). Application to in vivo LCTI data achieved plausible estimates (E: 0.49 MPa, γ: 8.12, τ: 8.34 s), consistent with observed displacement patterns.

**Conclusions:** This study successfully developed an VFM-based approach for in vivo ONH viscoelastic characterization using physiological ocular pulse loading. This offers potential for the non-invasive clinical assessment of ONH biomechanics, potentially aiding personalized glaucoma risk assessment and early detection.

## OCT STRUCTURAL CHANGES CORRELATE WITH RETROLAMINAR OPTIC NERVE ALTERATIONS IN NON-HUMAN PRIMATE EXPERIMENTAL GLAUCOMA

**Hongli Yang, Galen Willams, Jason Zhao, Jorie Liang, Juan Reynaud, Claude Burgoyne, Priya Chaudhary**

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We use immunohistochemistry (IHC) to study how protein alterations correlate with optical coherence tomography (OCT) structural change in non-human primate (NHP) early experimental glaucoma (EG). This study tests the hypothesis that retrolaminar 2',3'-Cyclic-nucleotide 3'-phosphodiesterase (CNPase) and glial fibrillary acidic protein (GFAP) expression correlate with longitudinal structural change in EG.

24 eyes from 12 NHPs with unilateral EG were included in this study. For each eye, longitudinal OCT peripapillary retinal nerve fiber layer thickness (pRNFLT), pRNFL tissue reflectance (pRNFLR) and minimum rim width (MRW) were quantified within 30° sectors. Four paraffin embedded sections per eye were deparaffinized and stained with CNPase and GFAP. IHC intensities were measured in each section within Band 1 (0-200µm posterior to the lamina cribrosa) and Band 2 (200-400µm). IHC section was colocalized to a fundus image and sectoral locations were estimated based on shared anatomy. EG vs. Control IHC intensity percent differences were correlated with longitudinal percent change of pRNFLT/pRNFLR/MRW in the same OCT sector using general estimation equation models.

Median percent inter-eye intensity differences were -57.4% for CNPase (range -87.2% to -58.8%), and -12.5% for GFAP (-52.6% to 186.9%), in 12 EG eyes showing mild pRNFLT loss (< 40%). pRNFLT, pRNFLR and MRW reduction was significantly correlated with CNPase intensity loss, and MRW change significantly correlated with GFAP intensity changes at the same sectors within both bands (all $p < 0.05$).

Our data shows that protein differences were related to longitudinally-detected pRNFLT, pRNFLR, and rim change in early EG. This study provides evidence that pathologic processes occur early in NHP EG eyes with minimum ONH structural changes. Linking biological mechanisms to ONH structural change will enhance the interpretation of OCT findings and inform our understanding of the pathophysiology of glaucoma.

Acknowledgements: Bright Focus and Legacy Good Samaritan Foundations (HY/PC), R01 EY011610 (CFB), ATL3DSuite software (Dr. Brad Fortune).

## AXONAL INJURY FROM REPEAT AWAKE CONTROLLED ELEVATIONS OF INTRAOCULAR PRESSURE IN ADULT AND AGED RATS

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We recently developed a Pinport/microcannula system to elevate IOP to a well-defined level and duration in awake rats. This eliminates the need for general anesthesia and allows us to model glaucoma as a series of repeat, awake-controlled elevations of IOP (aCEI). Here, we evaluated axonal injury following aCEI in adult (9-month-old; N = 17) and aged (24-month-old; N = 13) rats. Using the Pinport/microcannula system, animals underwent one or four 7-hour aCEI exposures, at an IOP of 45 mmHg (approximately twice the mean daily rat IOP). Optic nerves were collected 1-2 weeks after the last aCEI, embedded in plastic, sectioned, and graded on a scale from 1 (no injury) to 5 (> 50% axons degenerating) by 3 masked observers. Mean injury grades were analyzed using one-way ANOVA followed by Tukey's multiple comparisons test and compared to contralateral nerves from 8 adult and 9 aged rats. Mean TonoLab IOP during aCEI did not significantly differ ($p = 0.4$) between adult (40 ± 2 mmHg) and aged (44 ± 5 mmHg) rats. In adult rats, axonal injury did not change after just 1 aCEI (1.15 ± 0.30; $p = 0.1$) when compared to adult control eyes (1.04 ± 0.08). However, injury in adult rats significantly increased after 4 aCEI (1.74 ± 0.94; $p = 0.04$), relative to adult control eyes. In aged rats, injury after 1 aCEI (1.39 ± 0.39; $p = 0.02$) and 4 aCEI (1.98 ± 1.17; $p = 0.01$) were significantly increased relative to aged controls (1.04 ± 0.11). There was no significant difference in axonal injury between adult and aged rats after 4 aCEI ($p = 0.52$). These results show that multiple 7-hour awake CEI exposures can generate significant axonal injury in both adult and aged rats. The aCEI model, unencumbered by long-term anesthesia, allows accurate correlation between level and duration of IOP exposure and the resulting cellular responses. With this, we can learn how these events cumulatively contribute to axonal degeneration and blindness in chronic glaucoma.

## PRIMARY CILIUM REGULATES NITRIC OXIDE PRODUCTION IN SCHLEMM'S CANAL

**Myoungsup Sim[1], Aleks Grimsrud[1], Kevin Betsch[1], Mi Sun Sung[1], Vaibhav Desikan[1], Emrah Eroğlu[2], Paloma Liton[1]**

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Intraocular pressure (IOP) homeostasis is essential for preventing glaucoma. Our previous studies identified the primary cilium (PC) as a mechanosensor mediating mechanical stress-induced autophagy in IOP regulation. Meanwhile, nitric oxide (NO) production in Schlemm's canal (SC) has been reported to increase in response to shear stress and contribute to IOP control. To investigate whether the PC mediates NO production in SC, we developed a tamoxifen-inducible mouse model with SC-specific PC depletion ($IFT88^{fl/fl}$; Prox1-$CreER^{T2}$) and established a novel approach using genetically encoded NO probes (GeNOps) to visualize NO in vivo. We confirmed the presence of PCs in both SC and trabecular meshwork cells, with SC cilia protruding into the SC lumen. Tamoxifen treatment selectively ablated PCs in SC and significantly reduced NO levels, without altering endothelial nitric oxide synthase (eNOS) protein expression, which was the only NOS isoform detected in the iridocorneal angle. These findings were corroborated in vitro using EA.hy926 endothelial cells, where pharmacological deciliation reduced GeNOp-detected NO levels, which were restored upon reciliation. Our results identify the primary cilium as a critical regulator of NO production in SC and provide a new framework for enhancing NO-based therapies for glaucoma.

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## SEQUESTRATION OF ROCK1 BY CAVEOLIN-1 IN HUMAN SCHLEMM'S CANAL CELLS AFTER CYCLIC STRETCH

**Philip Mzyk[1], Michael Elliott[2], W. Daniel Stamer[1]**

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Intraocular pressure (IOP) homeostasis requires the cells of the conventional outflow pathway (trabecular meshwork, TM and Schlemm's canal, SC) to sense and respond to changes in IOP. Caveolae are abundantly expressed by SC and TM cells, functioning as mechanosensors and mechanoprotectors. Sequence variants in the caveolin-1 (CAV1) gene, that codes for the primary component of caveolae, reproducibly associate with risk for elevated IOP and POAG. Since mechanosensing by CAV1 involves protein scaffolding that is cell-type-specific, the goal of the present study was to define the molecular machinery in SC cells through which caveolae transduce changes in IOP.

To profile the mechanosensitive CAV1 interactome, we immunoprecipitated CAV1 and bound proteins from primary cultures of human SC cells (n = 7 different cell strains) that were subjected to cyclic stretch for 1 hour. Successful immunoprecipitation was confirmed by western blotting for CAV1, and bound proteins were analyzed by quantitative mass spectrometry. CAV1 interactions were defined by the stringent criteria of 2 or more peptides; ≥ 2-fold enrichment; and an ANOVA with $p < 0.05$. Bound proteins identified by mass spectrometry were validated by Western blot. Immunocytochemistry was also performed on non-stretched and stretched cells to examine localization of CAV1 binding partners.

We identified 81 CAV1-interacting proteins, of which 51 were uniquely found in stretched cells and 14 were shared between stretched and non-stretched cells. Bona fide caveolar proteins such as CAVIN3 and many cytoskeleton affecters in the Rho/rho-associated coiled-coil kinase-1 (ROCK) pathway were identified. For example, ROCK1 increased its association with CAV1 on average 46% (SD = 29.4) after cyclic stretch ($p = 0.04$). Importantly, CAV1 abundance was unchanged by exposure to cyclic stretch ($p = 0.85$).

Our study suggests that CAV1 serves to regulate SC contractility via sequestration of ROCK1 upon IOP elevation.

## CELL INTRINSIC DIFFERENCES IN ADHESIVE AND CONTRACTILE BEHAVIOR BETWEEN TM CELLS ISOLATED FROM SEGMENTAL FLOW REGIONS

**Souvik Ghosh[1], Rajanya Ghosh[1], Makenzie Miller[1], Lily Walker[1], VijayKrishna Raghunathan[2], Preethi Ganapathy[1], Samuel Herberg[1]**

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Aqueous humor outflow through the trabecular meshwork (TM) is non-uniform or segmental, with high-flow (HF) and low-flow (LF) regions. In the normal eye, LF-TM regions are more compact and stiffer compared to their HF-TM counterparts and are enriched in ECM proteins such as fibronectin. LF-TM regions become more numerous in glaucomatous eyes, which is thought to contribute to disease development. However, it is unclear why LF-TM regions exhibit distinct tissue characteristics. Here, we investigate whether normal donor-derived LF- and HF-TM cells (N = 3 paired cell strains) display intrinsic differences in contractile and adhesion machinery under identical biophysical conditions using the same soft ECM hydrogels. LF-TM hydrogels showed increased stiffness, deposition of total and EDA-fibronectin, and 3D contraction compared to HF-TM hydrogels ($p < 0.01$), remarkably consistent with the native tissue. This enhanced contractility of LF-TM cells was associated with increased actin stress fiber formation and α-actinin-mediated crosslinking compared to HF-TM cells ($p < 0.001$). Concurrently, LF-TM cells showed increased expression of Rho-associated kinase 1/2 as well as expression and phosphorylation of myosin light chain relative to HF-TM cells ($p < 0.01$), suggesting enhanced Rho/ROCK-driven cytoskeletal contractility and tension. Next, we investigated differences in key focal adhesion proteins that link the actomyosin cytoskeleton to the surrounding ECM via integrin transmembrane receptors. LF-TM cells showed increased clustering and size of αvβ3 integrins, concomitant with increased expression of talin, vinculin, and phosphorylated focal adhesion kinase compared to HF-TM cells ($p < 0.05$). This suggests that LF-TM cells form stronger adhesions with their surrounding ECM relative to HF-TM cells. Taken together, these data highlight cell intrinsic differences in adhesive and contractile behavior between segmental TM cells from healthy donors. LF-TM cells exhibit a distinctively more “activated” phenotype compared to HF-TM cells, suggesting that they may already be predisposed to more readily undergo pathological phenotypic conversion in glaucoma.

# PLATFORM SESSION 3
# GET REAL! - TRANSLATION

## 1:45 PM - 3:30 PM

## EMORY AMPHITHEATER

## SEEING THE DIFFERENCE: HOW AI AND OPTIC NERVE HEAD ATLASES SEPARATE GLAUCOMA FROM OPTIC NEUROPATHIES

**Thanadet Chuangsuwanich, Michael Girard**
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Differentiating glaucoma from other optic neuropathies remains a major diagnostic challenge due to overlapping structural features on OCT imaging. Leveraging Emory's large clinical OCT archive (> 330,000 scans from > 140,000 patients), we developed an AI-driven framework for constructing 3D optic nerve head (ONH) atlases across 10 major neuropathies and their stages. As a preliminary proof of concept, we applied this framework to a representative subset of the dataset to generate disease-specific atlases and compute localized strain maps using a novel unsupervised registration method (IDIR). Strain is defined here as the 3D spatial deformation required to morph an individual ONH scan to a reference atlas. It captures local differences in ONH structure – such as expansion, compression, or shear – between individuals and normative or disease-specific populations. These patterns serve as individualized structural fingerprints, with glaucoma typically showing posterior deformation (cupping) and other neuropathies exhibiting distinct patterns such as anterior swelling. Using Uniform Manifold Approximation and Projection (UMAP), we showed that these strain-derived features enable clear clustering of glaucomatous and non-glaucomatous optic neuropathies, including compressive, hereditary, ischemic, and inflammatory types. This work demonstrates that ONH strain maps can reveal subtle, disease-specific signatures not captured by conventional metrics, providing a strong foundation for anatomically grounded AI diagnostics capable of distinguishing glaucoma from its clinical mimics.

## PREDICTING FAST GLAUCOMA PROGRESSION: AN EXPLAINABLE MULTIMODAL GRAPH NEURAL NETWORK USING SINGLE-TIME-POINT OPTIC NERVE HEAD MORPHOLOGY AND VISUAL FIELD

**Patipol Tiyajamorn, Thanadet Chuangsuwanich, Yibo Chen, Deepta Ghate, Michaël Girard**
*Emory University, Department of Ophthalmology, Atlanta, United States*

We developed an explainable multimodal graph neural network (GNN) to predict fast glaucoma progression from a single clinical visit by fusing structural optic nerve head (ONH) morphology with functional visual field (VF) data. We analyzed 8,523 optical coherence tomography (OCT) scans from Emory Eye Center patients, labeling 2,075 as fast-progressors and 6,448 as slow-progressors using the Humphrey Visual Field Analyzer (HFA) Glaucoma Progression Analysis (GPA), where visual-field-index slopes of -1%/year or less are classified as fast glaucoma progression. Each eye's earliest scan was automatically segmented into 7 major neural and connective tissues using Reflectivity software. 32 radial slices centering on the Bruch's membrane opening (BMO) are extracted, converted into 2D graphs by linking nodes and edges along the tissue boundaries, and linked circumferentially to yield a 3D ONH graph with 7,104 nodes and 28,928 edges. Position, bordering-tissue labels, and local tissue thickness are encoded within each node, and paired-node information, distance, and orientation are encoded within each edge. The corresponding 24-2 pattern-deviation VF, obtained from the HFA, was flattened, encoded, and appended to every node, creating a fully multimodal graph. The ONH graph is convolved using Graph Attention Networks v2 layers, followed by a multilayer perceptron to provide glaucoma prognosis. With an 80/20 train-test split, our model achieved good performance with 0.76 area-under-the-curve (AUC) and 0.76 accuracy. Applying SHAP and Integrated Gradients reveals the center of the lamina cribrosa and internal limiting membrane, followed by the inferior-temporal regions of the sclera and Bruch's membrane as the most influential landmarks. This single-visit structure-function GNN delivers accurate glaucoma progression predictions and serves as a foundational model of the ONH for integrating additional data such as biomechanics and flow, while providing explainability for prognostic assessments.

## INCREASED PULSATILITY IN PERIPAPILLARY ARTERIES IS ASSOCIATED WITH MORE RAPID DISEASE PROGRESSION IN PRIMARY OPEN-ANGLE GLAUCOMA

**Stuart Gardiner, Grant Cull, Juan Reynaud, Hongli Yang, Steve Mansberger, Brad Fortune**
*Devers Eye Institute, Discoveries In Sight, Portland, United States*

Several hypothesized aspects of glaucomatous pathophysiology likely alter the vasodilatory capacity of vessels in the optic nerve head and/or retina, including biomechanical stiffening, upregulated Endothelin-1 system, and aberrant pericyte activation. Reduced vasodilation causes increased pulsatility upstream, and a decrease downstream. This project aims to identify the timing and location of changes in pulsatility, to infer the timing of those aspects of pathophysiology relative to axon loss. We applied a Deep Learning model to OCTA scans to identify perpendicular cross-sections of peripapillary vessels 500 μm outside Bruch's Membrane Opening, and classify them as arteries or veins; then colocalized these to Laser Speckle Flowgraphy (LSFG) scans, comprising 30 frames per second for 4 seconds. Pulsatility along these cross-sections was calculated as the maximum power of the Fourier-transformed pulse cycle divided by mean flow at each pixel, minus the choroidal signal measured at pixels up to half a vessel width outside the vessel along the same cross-section. Average pulsatility was calculated for all peripapillary arteries, and restricted to the superior or inferior hemifield, for series of 6 biannual test dates in 139 eyes of 181 participants with glaucoma / suspected glaucoma. Globally, higher arterial pulsatility was correlated with more rapid functional loss (Mean Deviation from automated perimetry: correlation $r = -0.220$, $p = 0.004$ [GEE regression]) and structural loss (Retinal Nerve Fiber Layer Thickness from OCT: $r = -0.220$, $p = 0.006$). Pulsatility in superior peripapillary arteries was non-significantly more strongly correlated with the rate of functional loss in the inferior hemifield ($r = -0.189$) than the superior hemifield ($r = -0.126$). Similarly, pulsatility in inferior arteries was non-significantly more strongly correlated with the rate of functional loss superiorly ($r = -0.251$) than inferiorly ($r = -0.198$). Our results suggest reduced vasodilatory capacity downstream of the peripapillary retina (i.e. not just in the optic nerve head), possibly localized to the hemifield experiencing rapid progression.

## QLS-111 SAFELY AND EFFECTIVELY LOWERED INTRAOCULAR PRESSURE IN TWO PHASE 2 CLINICAL TRIALS, OSPREY AND APTERYX

**Barbara Wirostko[1], Lisa Brandano[2], Thurein Htoo[1], Uttio Roy Chowdhury[2], Ralph Casale[1], Daniel Dewalt[2], Laura Rykard[1], Janet Serle[3], Bac Nguyen[4], Arthur J. Sit[5], Michael Fautsch[5]**

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Lowering episcleral venous pressure (EVP) offers an innovative approach for reducing intraocular pressure (IOP) in patients with glaucoma and ocular hypertension (OHT). QLS-111, a novel ATP-sensitive potassium channel opener formulation, lowers IOP by enhancing distal outflow through vasorelaxation and directly reducing EVP. Two randomized, controlled, double-masked Phase 2 studies evaluated the safety, tolerability, and efficacy of QLS-111 at three concentrations with two dosing regimens in patients with primary open-angle glaucoma (POAG) or OHT.

In Osprey (NCT06016972), 62 patients were washed out of IOP-lowering therapies and randomized to QLS-111 (0.015%, 0.030%, or 0.075%) or vehicle for 21 days. Dosing progressed from once-daily morning (QAMx7days) to evening (QPMx7days) and then twice daily (BIDx7days). In Apteryx (NCT06249152), 32 patients controlled on latanoprost with IOP ≥ 19 mmHg received QLS-111 or vehicle QPM for 14 days, then BID for 14 days. IOP was measured at 3 time points and safety was assessed via adverse events (AEs), vital signs, and ophthalmic exams.

Participants (52% male) averaged 67.3 years of age. In Osprey, QLS-111 at 0.015% and 0.030% produced significant mean diurnal IOP reductions. Baseline IOPs were 23.0 mmHg (0.015%), 23.7 mmHg (0.030%), 24.1 mmHg (0.075%), and 24.2 mmHg (vehicle). QLS-111 (0.015%) lowered IOP by 2.8 mmHg (QAM, $p = 0.0018$), 3.7 mmHg (QPM, $p = 0.0001$), and 2.8 mmHg (BID, $p = 0.0018$). In Apteryx, 0.015% QLS-111 concomitant with latanoprost reduced IOP by 3.2 mmHg (QPM, $p = 0.0005$) and 3.6 mmHg (BID, $p = 0.0002$) from a baseline of 19.8 mmHg. No serious AEs were reported in either study. Mild hyperemia and irritation occurred only in higher-dose (0.075%) and vehicle groups. No treatment-related AEs were seen with 0.015%.

QLS-111 was well tolerated and effectively reduced IOP in POAG and OHT, showing significant additive effects with concomitant latanoprost. Further development of QLS-111 as monotherapy or fixed dose combination, including for normal tension glaucoma is currently underway.

## PER-001 INTRAVITREAL IMPLANT (SUSTAINED RELEASE ENDOTHELIN RECEPTOR ANTAGONIST) DEMONSTRATED ≥ 7 DB VISUAL FIELD GAINS IN SUBJECTS WITH PROGRESSIVE GLAUCOMA

**Phillip Lai[1], Heather Schmitt[1], Michelle Chen[1], Dale Usner[2], Sevgi Gurkan[1]**
*[1]Perfuse Therapeutics, South San Francisco, United States, [2]Ophthalstat, Athol, United States*

Impaired blood flow to the optic nerve head plays a major role in glaucoma pathophysiology. Endothelin-1 (ET-1), the most potent endogenous vasoconstrictor is elevated in glaucoma and contributes to vascular dysregulation. PER-001 is an ET-1 receptor antagonist in a bioerodible intravitreal (IVT) implant designed to release PER-001 over 6 months. We conducted a 6 month, randomized, patient and reading center masked, Phase 2a study evaluating the safety, pharmacodynamics, and exploratory efficacy of a single dose of PER-001 in subjects with progressive mild-moderate glaucoma on standard of care IOP lowering therapies. Subjects were randomized to low dose (n = 9), high dose (n = 8) of PER-001 or a sham control (n = 8) group. Visual field (VF) sensitivity changes were assessed in six predefined areas based on Garway-Heath sectors that included a minimum of 5 measurable VF test points. A clinically meaningful change was defined as a mean ≥ 7 dB improvement or ≥ 7 dB worsening from baseline in any predefined sector over the 6-month study. A mean ≥ 7 dB improvement in at least one sector was observed in 3/8 subjects (37.5%) in the high dose group and 2/9 subjects (22.2%) in the low dose group compared to 0/8 subjects (0%) in the control group. In contrast, a mean ≥ 7 dB worsening occurred in 1/8 (12.5%) of the control group, 0/9 (0%) in the low dose group and 0/8 (0%) in the high dose group. In addition to the positive topline results reported recently (ARVO 2025), these findings demonstrate that PER-001 IVT treatment led to clinically meaningful improvement in VF in subjects with progressive glaucoma. A mean ≥ 7 dB visual gain aligns with the FDA's guidance on definition of clinical significance, supporting PER001's potential as a first-in class, IOP-independent, disease modifying treatment for patients with glaucoma.

## A NOVEL DEPLOYABLE MICROSTENT FOR THE TREATMENT OF GLAUCOMA

**Jared Ching[1], Yunlan Zhang[1], Weijia Zhang[2], Yunfang Yang[1], Callum Cuttle[1], Liam Morrow[1], Chun Zhang[2], Zhong You[1]**

*[1]University of Oxford, Oxford, United Kingdom, [2]Tsinghua University, Beijing, China*

In order to address the shortcomings of commercially available minimally invasive surgical approaches targeting the subconjunctival space for glaucoma, we developed a novel deployable microstent fabricated with nickel titanium (nitinol). By optimising the micro- and macro-geometry, this microstent mechanically supports the subconjunctival space at the terminus of a flexible helical tube. Leveraging the superelastic properties of nitinol and principles of kirigami, we were able to utilise precision machining to develop new prototypes. Theoretical and numerical models guided these designs, permitting optimal flexibility and hydraulic resistance for intraocular use. Ex vivo surgical implantation using cadaveric porcine eyes confirmed intraocular pressure (IOP) reduction and cadaveric human implantation guided structural refinement. In vivo implantation in rabbit models revealed that IOP can be lowered over 28 days with minimal inflammation and fibrosis on histological examination. We then undertook a head-to-head experiment with the commercially available XEN® gel stent without subconjunctival fibrosis augmentation and found that IOP did not change significantly after 14 days with XEN®. In contrast, following implantation of the novel microstent, a significant reduction in IOP was found at 28 days (-18.23%) and 42 days (-17.10%). Flow studies using fluoresceine sodium demonstrated subconjunctival flow appearing to be traverse more posteriorly than that of the XEN and with a smaller area of fluorescence. To date, this is the first deployable microstructure designed to treat glaucoma, demonstrating potential to provide long term IOP control beyond what is currently available. Further, our results indicate that such a design may mitigate the need for anti-fibrotic agents.

## EXTENDED INTRAOCULAR PRESSURE (IOP) REDUCTION BY HYDROGEL INJECTION INTO THE SUPRACHOROIDAL SPACE

**Ross Ethier[1], Yoree Chung[2], Zachary Thomas[3], David Ku[4], Daniel Ng[3], Shinyoung Park[3], Shan Fan[5], Carol Toris[6], Mark Prausnitz[3]**

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Microneedle injection of a hydrogel into the suprachoroidal space (SCS) reduces IOP for up to 4 months in rabbits and 1 month in nonhuman primates, providing a non-surgical, drug-free treatment for glaucoma. To increase treatment duration and improve translational potential of this method, we evaluated the safety and efficacy of two longer-lasting hydrogels for sustained IOP reduction after a single injection. We also conducted studies to elucidate the mechanism of IOP-lowering.

A single microneedle SCS injection of either a hyaluronic (HA)-based (biodegradable, n = 6 eyes) or synthetic (nondegradable, n = 7 eyes; MDInnovate, Inc., Decatur, GA) hydrogel was performed in normotensive New Zealand rabbits; naïve eyes (n = 3) served as controls. We measured IOP via tonometry and SCS expansion via ultrasound biomicroscopy. Safety evaluations were performed with exterior and slit-lamp examination, fundus imaging, electroretinography (ERG), optical coherence tomography (OCT), and histologic analysis. To determine the mechanism of IOP-lowering, we tracked a fluorescein-conjugated dextran tracer as a surrogate measure of unconventional outflow and measured aqueous inflow by fluorophotometry.

The biodegradable formulation expanded the SCS and reduced IOP for one year. The nondegradable formulation maintained SCS expansion and IOP reduction for over one year (throughout two years in n = 2 eyes). Clinical examinations showed the injections were well tolerated; histological analysis throughout one year revealed localized, stable fibroplasia without compromising retinal function (ERG). Mechanistically, we observed increased tracer penetration into the unconventional pathway at gel injection sites, and calculations showed that most of IOP lowering was due to increased unconventional outflow. Hydrogel injection into the SCS can provide sustained IOP reduction for more than one year after a single injection with a favorable safety profile for management of glaucoma.

Acknowledgements: Georgia Research Alliance, BrightFocus Foundation (G 2022013S) and NIH (F30EY035173)

# YOUNG INVESTIGATORS PLATFORM SESSION 4 THE NEXT GEN-EYE-RATION

## 8:45 AM - 10:05 AM

## EMORY AMPHITHEATER

## COL1A1 DEFICIENCY CAUSES CHANGES IN THE ULTRASTRUCTURE OF THE CORNEOSCLERAL SHELL AND LOSS OF RETINAL GANGLION CELLS AND THEIR AXONS

**Johanna Heimbucher[1], Ester Reina-Torres[2], Mara Imdahl[1], Matthias Mack[3], Darryl R. Overby[2], Ernst R. Tamm[1], Magdalena Gebert[1]**

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Biomechanical properties of the corneoscleral shell may modulate the susceptibility of optic nerve (ON) axons to glaucoma-related injury. To test this hypothesis we investigated mutant mice with a heterozygous deficiency in COL1A1 (UbiCre3 Col1a1$^{wt/fl}$). COL1A1 is the main component of collagen I, the major structural fibrillar protein of sclera and cornea.

Col1a1 reduction was confirmed by RT-PCR and Western blot. Ocular morphology was assessed by light microscopy, while collagen fibrils were quantified in TEM images of meridional sections. Myelinated ON axons were counted in PPD-stained ON cross-sections, whereas retinal ganglion cell (RGC) somata were quantified in retinal wholemounts immunolabelled against RBPMS. Intraocular pressure (IOP) was measured via rebound tonometry and adjusted for potential changes in corneal properties. Ocular compliance was measured using the iPerfusion system and subsequently analyzed using the Discrete Volume Method.

Mutant mice had a normal life span, a transparent cornea and did not show an obvious ocular phenotype. However, sclera and central cornea were significantly thinner when compared to control littermates. Also diameters of individual scleral and corneal collagen fibers were significantly thinner. Scleral collagen fibers were more densely arranged while corneal fibril density remained unchanged. ON axon number in 2- and 4-month-old mutant mice did not differ from their control littermates. However, by 5 months of age, a significant reduction in ON axons was observed, which correlated with a significant decrease in RGC somata. Ocular compliance of 3-month-old mice did not differ from that of control littermates, while IOP was significantly lower in COL1A1-deficient mice.

A thinner corneoscleral shell with an unchanged ocular compliance suggests an increased scleral elastic modulus in mutant mice. The increased elastic modulus may be the essential factor causing progressive loss of ON axons and RGC somata, even under conditions of low IOP. Similar mechanisms may contribute to glaucomatous damage in humans.

## DIVERGENT NUCLEAR MECHANOTRANSDUCTION PROCESSES IN HUMAN SEGMENTAL TM CELLS

**Souvik Ghosh[1], Rajanya Ghosh[2], Ester Reina-Torres[3], Haiyan Gong[4], Vijay Raghunathan[5], Darryl Overby[3], Preethi Ganapathy[6], Samuel Herberg[2]**

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The trabecular meshwork (TM) exhibits a segmental pattern of aqueous humor outflow, with high-flow (HF) and low-flow (LF) tissue regions of differential stiffness. While understanding of TM mechanobiology is evolving, little is known about specific mechanotransduction differences in HF- and LF-TM cells, in part, due to the scarcity of isolated cells for mechanistic studies. The nucleus is now widely seen as an active mechanosensor, converting extracellular mechanical signals into structural/transcriptional responses. Here, we investigate whether TM cells derived from HF- and LF-regions (N = 3 paired cell strains) display intrinsic differences in nuclear mechanotransduction under identical biophysical conditions using soft ECM hydrogels. LF-TM cells showed increased nuclear volume compared to HF cells ($p < 0.001$), with a similar nuclear phenotype also found in conventional 2D cultures on glass and in situ by serial block-face scanning electron microscopy of human segmental TM tissue. LF-TM cells displayed increased expression of LINC complex components Nesprin-2 and SUN1 compared to HF-TM cells ($p < 0.001$), suggesting increased mechanical coupling between the cytoskeleton and nuclear interior. Similarly, Lamin A/C levels were higher in LF- vs. HF-TM cells, with smoother nuclear lamina indicative of laminar adaptation to sustained intracellular tension. HF-TM cells displayed concentrated perinuclear Emerin and elevated HP1α expression ($p < 0.001$), suggesting a closed heterochromatin state. In contrast, we noted diffuse Emerin distribution and increased levels of the euchromatin marker H3K9/14ac in LF-TM cells, indicating an open euchromatin state. While total HAT activity was comparable between groups, total HDAC activity was decreased in LF- vs. HF-TM cells ($p < 0.01$), with HDAC3 as the major differentially expressed isoform. Together, these data demonstrate that segmental TM cells preserve region-specific nuclear mechanobiological signatures, and that such divergence may underlie localized TM dysfunction in glaucoma. We identify the nuclear envelope and chromatin-modifying enzymes as potential therapeutic targets for modulating segmental TM mechanotransduction to restore physiological outflow function.

## CELL-BASED INSULIN DELIVERY PROMOTES RETINAL GANGLION CELL REGENERATION AND RESTORES VISUAL FUNCTION IN GLAUCOMA

**Sana El Hajji[1], Clara Goubault[2], Yukihiro Shiga[1], Renata Ghenno Manrique[1], Nicolas Belforte[1], Melanie Ethier[2], Isaac Vidal Paredes[1], Florence Dotigny[1], Vincent Poitout[2], Adriana Di Polo[1]**
*[1]CRCHUM, Neuroscience, Montréal, Canada, [2]CRCHUM, Montréal, Canada*

Topical insulin has been shown to exert a powerful pro-regenerative and neuroprotective effect in mouse and non-human primate glaucoma models, and this strategy is currently being tested in human clinical trials for this disease. Insulin eyedrops present challenges including reduced biological activity and poor patient compliance. Here, we explored a novel approach based on the transplantation of islets of Langerhans (IL) into the anterior chamber. This location offers advantages such as high oxygen tension, immune privilege, and high optical transparency for live imaging of islet cells.

Donor mice pancreas were obtained followed by IL isolation and injection into the anterior chamber of recipient mice (~50 islets/eye). Insulin levels in the retina were measured by ELISA andblood glucose levels in host mice were monitored weekly. IL vascularization was visualized after retroorbital injection of fluorescent dextran. RGC dendrites were imaged in Thy1-YFP mice after magnetic microbead injection to induce ocular hypertension (OHT) and IL transplantation. Light-evoked single-RGC calcium (Ca2+) dynamics were recorded using two-photon microscopy in Thy1.GCaMP6f mice. Optomotor responses were assessed weekly post-transplantation.

IL grafts rapidly attached to the iris, became vascularized, and remained stable for several weeks. Insulin levels in the retina increased starting at 2 weeks after transplantation and remained elevated thereafter ($p < 0.0001$). We found robust RGC dendrite regeneration and survival after IL transplantation relative to sham-operated mice ($p < 0.0001$). IL grafts increased the density of RGC excitatory postsynaptic sites in both ON and OFF neurons. Furthermore, host mice displayed a substantial recovery of light-evoked $Ca2^{+}$ dynamics ($p < 0.05$) and improved visual acuity measured through optomotor responses ($p < 0.001$). IL transplantation did not change IOP or blood glucose levels.

IL transplantation is an effective strategy for sustained insulin delivery resulting in successful RGC regeneration, neuroprotection, and functional recovery during glaucomatous stress.

Funding: The Glaucoma Foundation.

## AI-BIOMECHANICAL 3D MAPPING OF OPTIC NERVE HEAD REMODELING AND AXONAL LOSS IN GLAUCOMA

**Yibo Chen, Thanadet Chuangsuwanich, Patipol Tiyajamorn, Michaël Girard**
*Emory University, Department of Ophthalmology, Atlanta, United States*

We aimed to modify Implicit Neural Representations for Deformable Image Registration (IDIR) to map optic Nerve head (ONH) structural remodeling, including axonal atrophy, in response to glaucoma progression. This model, integrating biomechanical principles with deep learning, can rapidly and accurately establish one-to-one mapping between sequential 3D optical coherence tomography (OCT) scans and quantify biomechanical properties, revealing remodeling patterns. The model was validated on artificial datasets (non-rigid ONH deformation to mimic real intraocular pressure), with its robustness to noise and consistent performance across varying parameters evaluated. For real-world application, we utilized longitudinal OCT scans from 45 glaucoma eyes (follow-up: 7.02 ± 2.61 years) sourced from Emory Eye Center's retrospective cohort over the last 15 years. Prior to analysis, 3D ONH tissue segmentation (acquired from OCT scans by Abyss Processing Reflectivity ) was performed. We evaluate the model performance by comparing the morphed segmentation and target segmentation, and the method achieved a high DICE coefficient (0.8049 ± 0.0335), while maintaining a short processing time (4.42 ± 0.04 min), significantly outperforming conventional Digital Volume Correlation (DVC) methods, which take hours. Comparative analysis of longitudinal strain maps in the same patient revealed accumulating strain in the retinal nerve fiber layer (RNFL) and prelamina, indicating chronic tissue remodeling. Furthermore, when contrasted with RNFL thickness reduction, our strain maps not only delineated compromised RNFL areas but also presented a rim-like high-strain pattern within the optic disc. This technique enables fast, quantitative analysis of ONH biomechanics and offers greater clinical applicability than DVC, providing valuable insights into tissue remodeling and axonal loss, and potentially detecting early progression missed by standard RNFL thickness measurements.

# MICROTUBULE STABILITY MODULATES SCHLEMM'S CANAL CELLMECHANOBIOLOGY AND TRANSCELLULAR PORE FORMATION

**Haiyan Li[1], Kristin Perkumas[2], W. Daniel Stamer[2], Ross Ethier[1]**

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Schlemm's canal (SC) cells are mechanosensitive, as evidenced by their enhanced transcellular pore (I-pore) formation in response to mechanical stretch, as well as their ability to alter mechanobiological properties in response to substrate stiffness. In this study, we focus on the role of microtubules (MTs) in SC cells, given their mechano-responsive properties and evidence that they modulate intraocular pressure. Specifically, we investigated how MT stability modulates SC cell mechanobiology and I-pore formation, and how substrate stiffness affects MT dynamics.

Using primary human SC cells, we found that a 30-minute treatment with the MT stabilizer paclitaxel (10 μM) or the destabilizer nocodazole (10 μM) significantly increased and decreased MT acetylation, respectively – an established marker of MT stabilization. MT stabilization reduced phosphorylated myosin light chain levels by 58.3% ($p < 0.01$) and decreased cell stiffness (from 1.7 ± 0.7 kPa to 1.1 ± 0.5 kPa, $p < 0.01$), as measured by atomic force microscopy using a 10 μm spherical probe. In contrast, MT destabilization had the opposite effects. Interestingly, MT stabilization did not alter F-actin, while MT destabilization significantly increased F-actin levels (2.4-fold, $p < 0.0001$). Interestingly, we observed that MT stabilization-dependent modulation of SC cell mechanobiology was attenuated by siRNA-mediated knockdown of GEFH, a MT-associated guanine nucleotide exchange factor. Furthermore, cells cultured on soft biomimetic hydrogels (2.4 kPa) exhibited significantly higher MT acetylation than those on stiff substrates (8.0 kPa; $p < 0.05$), suggesting that substrate stiffness modulates MT stability. Functionally, MT stabilization enhanced I-pore formation in response to localized stretch induced by 5 μm-diameter microspheres positioned beneath the cells, as indicated by increased pore formation at microsphere-associated sites (16.4 ± 1.7% vs. 20.3 ± 2.2%, $p < 0.01$).

Together, our data suggest that MT stability modulates the actomyosin cytoskeleton, cell stiffness, and I-pore formation in SC cells, thereby potentially modulating conventional outflow resistance. These findings support MT stabilization in SC endothelial cells as a potential therapeutic strategy to enhance outflow facility for glaucoma treatment.

## AAV2-HSPB1 ENHANCES THE SURVIVAL OF HUMAN STEM CELL-DERIVED RETINAL GANGLION CELLS FOLLOWING TRANSPLANTATION IN MICE

**Mihyun Nam, Shama Parween, Michael Ha, Jiwoo Suk, M. Natalia Vergara, Ram Nagaraj**
*University of Colorado School of Medicine, Ophthalmology, Aurora, United States*

Glaucoma is characterized by the progressive loss of retinal ganglion cells (RGCs), leading to irreversible vision impairment. While current treatments can slow disease progression, they cannot restore lost RGCs. As a result, stem cell-based transplantation strategies are being explored to replace degenerated RGCs and restore visual function in advanced stages of the disease. However, the limited survival of transplanted cells remains a significant challenge. To address this, we aim to enhance the survival of human induced pluripotent stem cell (hiPSC)-derived RGCs using a previously validated gene therapy encoding the small heat-shock protein HSPB1. HSPB1 inhibits stress-induced apoptosis, and our previous studies have shown that AAV2-mediated transduction of HSPB1 in RGCs protects against cell death in mouse models of glaucoma. In this study, we transduced hiPSC-derived RGC cultures with AAV2-HSPB1 to evaluate its potential to improve cell survival. Our results show that AAV2-HSPB1 effectively overexpresses HSPB1 in RGCs and confers protection against oxidative or pro-inflammatory stress induced by H2O2 or a cytokine mixture. Additionally, HSPB1 transduction significantly promoted neurite outgrowth in hiPSC-derived retinal organoids. We intravitreally transplanted either naïve or HSPB1-transduced RGCs into mouse eyes and observed significantly improved survival of the engineered RGCs at two weeks post-transplantation. Furthermore, the RGCs were more abundant in the ganglion cell layer after four weeks. Finally, transplantation of engineered RGCs one week after optic nerve crush resulted in increased survival of donor RGCs and partial preservation of pattern electroretinogram response compared to the naïve RGC transplant group. We conclude that HSPB1 transduction of hiPSC-derived RGC provides more resilient cells for transplantation, which may facilitate the development of cell-based therapies for glaucoma.

# PLATFORM SESSION 5
# REGENERATION & NEUROPROTECTION: I WILL SURVIVE!

## 10:45 AM - 12:30 PM

## EMORY AMPHITHEATER

## SIGNALING CROSSTALK WITHIN THE OPTIC NERVE SUPPORTS SUCCESSFUL AXON REGENERATION

**Matthew Veldman[1], Mengming Hu[2], Robert Newland[2], Wesley Speer[2], Hllda Jurkiewicz[2]**

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Unlike mammals, zebrafish fully regenerate their optic nerve (ON) and recover vision after injury. The exact signaling mechanisms mediating their efficient axon regeneration are undefined. We used a combination of spatial transcriptomics, laser capture microdissection RNA-seq, and bulk RNA-seq to reveal the differential expression of genes across the healthy and regenerating visual system. We have begun to test the role these identified genes and pathways play in ON regeneration. Tissue was harvested from adult zebrafish 3 days after optic nerve crush when regenerating axons are present in the ON. K-means clustering revealed 10 regions of differential gene expression across the ON and brain suggesting that the injury site and associated optic pathway have distinct regeneration-associated changes. The DanioTalk database was used to identify ligand-receptor pairs that may be mediating signaling between the retinal ganglion cell (RGC) axons and ON environment. kitb and pdgfra were identified as highly induced in RGCs after injury and their ligands, kitlgb and pdgfaa, upregulated in ON, tract, and tectum. In situ hybridization assays validated this finding. To test the functional relevance of identified genes we chose to inhibit the function of the Kitb and Pdgfra receptors using antisense morpholinos and chemical inhibitors. Axon regeneration into the brain was measured in control and treated groups using the gap43:GFP reporter line at 7 days post injury. Chemical or genetic inhibition of kitb or pdgfra resulted in significantly decreased regeneration which was enhanced by co-knock down suggesting redundancy in function. We are currently testing the functional role of candidate ligands for Kitb and Pdgfra in supporting ON regeneration, as well as additionally ligand-receptor pairs. Our results suggest complementary changes in RGC and ON cell gene expression for ligand and receptor genes support ON regeneration not seen in mammals.

## EXTRACELLULAR PROTEIN COMPONENTS OF AMPA RECEPTORS: EFFECTS ON RETINAL BIOLOGY AND CONNECTION TO GLAUCOMA

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AMPA receptors (AMPARs) are the major ionotropic glutamate receptors in neuronal tissues. The hetero- or homotetramers of four core subunits (GluA1-4) form the AMPAR complex along with about 25 other proteins. Available data suggest that the composition of AMPARs could be modified in retinal pathologies, including glaucoma. We investigated the role of several extracellular protein components of AMPARs (Olfactomedins or Olfm1-3, Neuritin or Nrn1, Brorin or Vwc2, and Brorin-like or Vwc2l) in retinal biology with a primary focus on retinal ganglion cells (RGCs) and glaucoma. Pattern electroretinogram (PERG) recordings of mouse knockout lines with deficiency in Olfm1, Olfm2, Olfm3, Nrn1, Vwc2, and Vwc2l demonstrated statistically significant reductions in the PERG amplitude in Nrn1, Vwc2, Olfm2, and Vwc2l KO retinas compared with wild-type (WT) retinas with Nrn1 KO retinas being the most affected. Olfm2 and especially Nrn1 were preferentially expressed in RGCs, while Vwc2 and Vwc2l were more actively expressed in cells located in the retinal inner nuclear layer. Above mentioned extracellular proteins may interact with each other and the core subunits of AMPARs with different specificity. Vwc2 interacts with Olfm2 with higher affinity compared with Olfm1 and Olfm3, and Olfm2 facilitates interaction of Vwc2 with AMPAR core subunits. A model of an Olfm2-Vwc2-GluA2 homotetramer complex was built using AlphaFold 3. Vwc2 KO demonstrated a decrease in the number of horizontal, amacrine, and αRGCs. In Vwc2 KO, the total levels of AMPAR core subunits GluA1-GluA4 did not change compared with WT, while the ratio of GluA2/GluA1 subunits was decreased leading to increased retinal permeability to Ca2+ previously observed in rodent glaucoma models. These data identify extracellular components of AMPARs as critical players affecting retinal physiology and suggest their involvement in glaucoma pathology.

This work was supported by the Intramural Research Programs of the NEI.

## SURVIVAL, MIGRATION AND MATURATION OF STEM CELL-DERIVED "REPLACEMENT" RETINAL GANGLION CELLS AFTER TRANSPLANTATION INTO GLAUCOMATOUS NONHUMAN PRIMATE RETINA

**Brad Fortune[1], Joseph Leffler[2], Kazuhiro Kurokawa[1], Aris Thanos[1], Grant Cull[1], Michaela Dunn[1], Kana Orihara[1], Priya Chaudhary[1], Morgan Nemeth[1], Carson Prosser[3], Cátia Gomes[3], Subramanian Dharmarajan[4], Yvonne Ou[4], Benjamin Sivyer[2], Jason Meyer[3]**

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Restoration of vision in persons blinded by glaucoma and other optic neuropathies may include transplantation of "replacement" retinal ganglion cells (RGCs) such as those derived from human induced pluripotent stem cells (hiPSCs). To date, we have performed vitreoretinal surgery to transplant a suspension of ~670,000 hiPSC-RGCs into the glaucomatous eye of each of N = 17 adult rhesus macaque monkeys with unilateral experimental glaucoma (EG). An additional monkey underwent sham transplantation into its severely EG eye and two additional monkeys each had control surgery in their control eye. For up to 10 weeks following surgery, we performed imaging using fluorescence scanning laser ophthalmoscopy and optical coherence tomography (fSLO/OCT, Spectralis), as well as by adaptive optics (AO) fSLO/OCT in a subset of animals. Our results demonstrate that survival of donor RGCs in vivo is dramatically enhanced when they are freshly isolated from organoids and prepared on site versus being isolated at a remote site, then frozen, shipped and thawed. We have also observed that eyes with more severe EG at the time of surgery have worse inflammation and more substantial disruption of retinal architecture following transplantation. Imaging by Spectralis and AO-fSLO revealed that donor RGCs survived for up to 10 weeks in vivo, migrated within the host retina, developed neurites (in some cases branching into an arbor resembling those of host native RGCs), and extended putative axons many millimeters from the soma. Targeted patch clamp recordings ex vivo showed that input resistance and spike threshold decreased with longer post-transplantation duration in vivo in a manner similar to donor RGCs seeded on healthy adult rhesus retinal explants. Immunohistochemistry demonstrated that donor RGCs, but not host retinal cells, stained positive for markers of human stem cell origin. With longer post-transplantation duration in vivo, a higher proportion of donor RGCs stained positive for RBPMS.

## RESTORING AIBP EXPRESSION IN THE RETINA PROVIDES NEUROPROTECTION IN GLAUCOMA

**Wonkyu Ju**
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Glaucoma is a neurodegenerative disease manifested by retinal ganglion cell (RGC) death and irreversible blindness. We have identified apolipoprotein A-I binding protein (AIBP) that controls excessive cholesterol accumulation and neuroinflammation in the retina by upregulating the cholesterol transporter ABCA1 and reducing TLR4 signaling and mitochondrial dysfunction. Here, we demonstrated that AIBP and ABCA1 expression were decreased, while TLR4, IL-1β, and the cholesterol content increased in the retina of patients with glaucoma and mouse models of glaucoma. Restoring AIBP deficiency by a single intravitreal injection of AAV protected RGCs and ameliorated visual dysfunction in experimental glaucoma. Conversely, AAV-mediated RGC-specific AIBP knockdown exacerbated RGC loss and visual dysfunction in a mouse model of glaucoma. Mechanistically, AAV-AIBP attenuated TLR4 and IL-1β expression and localization of TLR4 to lipid rafts, reduced cholesterol accumulation, and ameliorated visual dysfunction. Additionally, AAV-AIBP promoted mitochondrial complexity and function in Müller glia in vivo. Recombinant AIBP protein inhibited TLR4 and IL-1β activation and alleviated mitochondrial dysfunction in Müller glia in response to elevated pressure in vitro. These studies indicate that restoring AIBP expression in the glaucomatous retina reduces neuroinflammation and protects RGCs and Müller glia, suggesting the therapeutic potential of AAV-AIBP in human glaucoma.

## PROTEASOMAL CONTROL IS CRITICAL FOR RETINAL GANGLION CELL VIABILITY

**Ekaterina Lobanova[1], John Ash[2], Issam Al Diri[2], Kun-Che Chang[2], Shaohua Pi[2], Takaaki Kuwajima[2]**
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Nuclear factor erythroid-2-like 1 (Nfe2l1) is a ubiquitously expressed stress-responsive transcription factor that regulates multiple cellular processes, including the expression of proteasomes—the principal proteolytic machinery of the ubiquitin-proteasome system (UPS). Our previous work demonstrated that Nfe2l1 overexpression increases proteasome levels and supports retinal viability in a mouse model of photoreceptor degeneration caused by protein misfolding.

To define Nfe2l1's role in retinal homeostasis, we generated retina-specific Nfe2l1 knockout (KO) mice and performed functional, molecular, and transcriptomic analyses. Unexpectedly, despite its broad expression, Nfe2l1 inactivation led to selective loss of retinal ganglion cells (RGCs), revealing their pronounced vulnerability to UPS dysfunction. VIS-OCT imaging revealed loss of nerve fiber bundles, pattern electroretinography (pERG) demonstrated impaired RGC function, and histological analysis confirmed optic nerve atrophy and RGC degeneration in one-month-old mice. Markers of RGC development were unaltered in early postnatal stages, and CTB tracing showed no defects in axon guidance—together indicating that Nfe2l1 is essential for RGC viability but not for their specification or connectivity.

To directly assess UPS activity in vivo, we utilized a UPS reporter mouse line expressing a short-lived GFP fused to a degron ($Ub^{G76V\text{-}GFP}$), which accumulates when proteasomal degradation is impaired. Under basal conditions, minimal reporter accumulation was observed in wild-type retinas, reflecting intact proteasome function. However, following optic nerve crush the reporter accumulated robustly in RGCs, indicating stress-induced impairment of UPS activity.

This study reveals a critical and underappreciated dependence of RGCs on UPS-mediated protein degradation. It suggests that enhancing proteasome activity may offer a therapeutic strategy to preserve vision in diseases characterized by RGC degeneration. Understanding how Nfe2l1 regulates proteostasis in these neurons may enable the development of targeted neuroprotective interventions for optic neuropathies and other neurodegenerative conditions affecting the inner retina.

## AN AI APPROACH TO IDENTIFY AND CLASSIFY RETINAL SYNAPSES IN ANIMAL MODELS OF GLAUCOMA

**Luca Della Santina**
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Glaucoma causes progressive dysfunction of the retina, ultimately leading to blindness. Animal models have shown that inner retina synapses are dismantled early after IOP elevation. As synaptic disassembly spans across the entire retina, the conventional approach of sampling small regions to quantify residual synapses falls short in identifying large patterns of synapse loss an rearrangement. We have developed a novel approach based on AI to identify and quantify synaptic proteins for excitatory and inhibitory synapses, allowing fast, accurate and automated detection across large portion of the inner retina.

Transient IOP elevation was induced in mice by photocoagulation of the episcleral and limbal vessels in adult CD1 mice. Retinal excitatory and inhibitory synaptic were targeted using the postsynaptic proteins PSD95 and Gephyrin, labeled by immunohistochemistry in the entire retina or biolistic transfection in individual RGCs. A ground truth database of manually annotated synaptic proteins by consensus of two human experts, with a minimum of 8,000 synapses per condition. AI models were trained using Nvidia Quadro RTX 8000 GPU and MATLAB Deep Learning Toolbox.

Automatic detection of synaptic proteins was achieved for both inhibitory and excitatory synapses by training multiple classifier models (AlexNet, GoogleNet, ResNet-50, Xception). Among them, ResNet-50 trained using control data from biolistically transfected tissue was able to predict synaptic identity with high levels of sensitivity and specificity in control (0.94 ± 0.01 and 0.80 ± 0.08 respectively) and glaucoma (0.807 ± 0.019 and 0.808 ± 0.022, respectively) retinas.

Excitatory and inhibitory synapses can be effectively annotated using AI. Post-synaptic proteins can be detected from models trained on control RGCs, and the same models are able to annotate synapses in retinas from either control or IOP elevated eyes without retraining. This novel tool has the potential to be used across laboratories working on different animal models of glaucoma.

## IPSILATERAL OPTIC NERVE DAMAGE IS EITHER PROTECTIVE OR EXACERBATES GLAUCOMA IN THE CONTRALATERAL EYE DEPENDING ON THE SEVERITY OF THE INJURY

**Rob Nickells**
*Ophthalmology and Visual Sciences, University of Wisconsin-Madison, Madison, United States*

Purpose: Unilateral optic nerve damage induces reactive-like astrogliosis in the contralateral retina. It is not known if this response results in the generation of neurotoxic astrocytes that could lower the threshold of the contralateral retina to a damaging stimulus like elevated IOP.

Methods: DBA/2J mice were used as a glaucoma model. Unilateral optic nerve crush (ONC), or a sham surgery, was performed on the left eye at 8 months of age and the contralateral eye was evaluated for glaucomatous damage at 10.5 months. Glaucomatous retinal damage (RBPMS staining) and axonal damage was compared to naïve mice with natural progression of disease. IOPs were taken at 8, 9.25, and 10.5 months of age. Separately, C57BL/6J mice were subjected to unilateral ONC. Both eyes were evaluated for reactive astrogliosis including planned spatial transcriptomics at 7 days post injury.

Results: Astrocytes in the retinas of C57BL/6J mice exhibited reactive-like morphological changes in the damaged eye by 1 day post-injury and in both eyes by 3 days post-injury. Spatial transcriptomic analysis is currently underway. The 3 groups of DBA/2J mice (naïve – n = 18 eyes, sham – n = 15 eyes, ONC – n = 12 eyes) all had statistically similar IOP histories at the 3 time points examined ($p > 0.1$). ONC mice exhibited greater glaucomatous damage compared to both naïve and sham-treated mice (retina, Chi2 $p = 0.014$, optic nerve, $p < 0.00001$), while sham-treated mice exhibited a dramatic reduction in the amount of retinal damage ($p < 0.00001$), but no change in optic nerve damage relative to the naïve group ($p = 0.14$).

Conclusion: Unilateral optic nerve damage influences pathology of the contralateral eye in response to elevated IOP. If the damage is severe, IOP-induced pathology is greater in the contralateral eye. If the damage is sub-lethal, the RGCs of the contralateral eye, but not the axons, exhibit an increase in resistance to IOP-induced stress.

# PLATFORM SESSION 6
# WHAT YOU NEED TO KNOW ABOUT GLAUCOMA

## 1:45 PM - 3:30 PM

## EMORY AMPHITHEATER

## NYCHTHEMERAL RHYTHMS OF IOP FOLLOWING TRABECULAR MESHWORK (TM) LASER IN NONHUMAN PRIMATES (NHPS)

**J. Crawford Downs, Christina Nicou, Lisa Hethcox, Chester Calvert, Sonia Kuhn-Asif**
*Ophthalmology and Visual Sciences, University of Alabama at Birmingham, Birmingham, United States*

Intraocular pressure (IOP) plays a significant role in the pathogenesis of glaucoma and the NHP has been used extensively to study the disease, in which the TM is lasered in one eye to chronically elevate IOP and generate experimental glaucoma. This study aimed to determine if the IOPs measured during typical laboratory working hours represent the IOP changes induced by TM photocoagulation. IOP data were continuously collected using implanted wireless telemetry at 250 Hz on a 33% duty cycle (40s of every 2-min period) for 14 days periods both pre- and post-TM laser in three NHPs implanted with our TSE-Systems Stellar system. IOP telemetry sensors were calibrated every two weeks via anterior chamber cannulation manometry to establish inter-calibration drift, and data were corrected assuming linear drift between calibrations. Data were filtered for signal dropout, then averaged in 3-hour periods; the central 6-hour periods in the waking (9am-3pm) and sleeping (9pm-3am) periods were compared to quantify the nychthemeral rhythm in the pre-laser and post-laser periods. On average, pre-laser IOPs were 11.3 ± 2.3 and 14.6 ± 2.5 mmHg (mean ± SD) during the waking and sleeping periods, respectively. Mean post-laser IOPs were significantly higher ($p < 0.001$), at 25.6 ± 7.7 and 26.3 ± 4.7 mmHg during the waking and sleeping periods, respectively. Pre-laser sleeping IOPs were significantly greater than waking IOPs in all animals ($p < 0.001$), and this difference was significantly larger in the post-laser period in all NHPs ($p < 0.001$). Post-laser sleeping IOP was significantly higher in 2 of 3 NHPs, but significantly lower in one NHP ($p < 0.001$). Individual NHPs exhibit a wide range of IOP behavior after TM laser, including individual-specific differences in nychthemeral rhythm. Results suggest that snapshot IOP measurements obtained during working hours may not be sufficient to characterize IOP change in the NHP model of glaucoma.

## FUNCTIONAL CHARACTERIZATIONS OF CILIA-CENTOSOMAL AND CRYSTALLIN GENES IN PRIMARY CONGENITAL GLAUCOMA PATHOGENESIS

**Subhabrata Chakrabarti, Goutham Pyatla, Anil Mandal, Samir Bera, Ashish Mishra, Meha Kabra1, Seema Banerjee, Inderjeet Kaur, Rohit Khanna1**

*L V Prasad Eye Institute, Brien Holden Eye Research Centre, Hyderabad, India*

Primary congenital glaucoma (PCG) is attributed to developmental defects in the trabecular meshwork and anterior chamber angle. Genetic heterogeneity is the hallmark of PCG, but the known genes do not explain the overall molecular basis of this disease. Our earlier studies indicated co-occurrences of multiple loci in PCG. We aimed to functionally characterize the underlying mechanisms resulting due to genetic and physical interactions of multiple genes/proteins in PCG. Our cohort comprised clinically well characterized PCG cases (n = 600) and ethnically matched normal controls (n = 2500) that were screened through an integrated targeted and whole exome sequencing (WES) approach. Standard protocols of library preparations were followed along with data filtration and curation using GATK and VarSeq softwares. Additionally, pathogenicity scoring of rare variants (REVEL scores) and functional characterizations were assessed through co-immunoprecipitation followed by immunoblotting of the rare variants in appropriate cells. Genotype-phenotype corelations were undertaken on longitudinal intraocular pressure (IOP), corneal diameter (CD), cup to disc ratio (CDR) and photopic negative response (PhNR) data in cases with and without mutations. Deep sequencings revealed involvements of rare pathogenic variants in twelve cilia-centrosomal (16.81%, 95%CI, 13.96%-20.11%) and eleven crystallin (2.79%, 95%CI, 1.47%-5.23%) genes in PCG cases. Additionally, co-occurring heterozygous alleles in DNAH11, GLIS1, TEK, RPGRIP1, CEP164, INPP5E, TBK1, MYOC, TGFb2 and CRYAB along with the PCG-associated candidate genes (CYP1B1, FOXC1) indicated that 6.37% (95%CI, 4.64%-8.69%) cases were either digenic, or had multi-allelic inheritance. Corresponding physical interactions were observed for CEP164 and TEK with CYP1B1 alleles in HEK293 cells. Genotype-phenotype correlation indicated poor prognosis in patients harbouring multiple alleles and the PhNR parameters suggested reduced retinal ganglion cell activity. Our data provided strong evidence for the involvement of cilia-centrosomal and crystallin genes in PCG and recommend further explorations of this yet unidentified molecular mechanisms in disease pathogenesis.

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## TOWARD MOUSE MODELS OF EXFOLIATION GLAUCOMA

**Michael Anderson[1], Kacie Meyer[2]**

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Exfoliation syndrome (XFS) is a systemic disease of elastin-rich tissues involving a deposition of fibrillar exfoliative material (XFM) in the anterior chamber of the eye. Despite XFS being a leading cause of secondary glaucoma worldwide, much remains unknown concerning its pathophysiology. To empower progress, we have been engaged in work to develop new mouse model of XFS. Our primary approach has been to manipulate candidate genes identified from human GWAS in mice via creating CRISPR/Cas9-induced variations and screen them for indices of XFS. Among 277 mice among 12 strains with overt mutations in *Agpat1*, *Cacna1a*, *Loxl1*, *Rbms3*, *Sema6a*, and *Tlcd5* genes of C57BL/6J aged to 5+ months, 902 slit-lamp exams failed to detect indices of XFS. We have also analyzed a BAC transgenic strain in which *LOXL1-AS1* was expressed. Among 37 mice aged to 5+ months, 136 slit-lamp exams failed to detect indices of XFS. Finally, we analyzed a new CRISPR/Cas9-induced mutant allele of Lyst, which is relevant to XFS because mice with the *bg-J* mutation have previously been shown to exhibit a pattern of iris transillumination defects unique to human XFS. The new mutant allele did not develop iris transillumination defects or XFS. In sum, these studies did not detect XFS in any of the strains. This may have been due to species-specific differences, background dependence, or insufficient aging. Alternatively, it is possible that the current candidates, selected based on proximity to GWAS signals, are insufficient as single effectors. Finally, in addition to the slit-lamp data above, we have also carefully characterized the appearance of the lens in C57BL/6J mice and will show two phenotypes which could readily be misinterpreted in the ongoing search by the field for mouse models of XFS (a “specular cataract” congenitally present in all eyes and a temporary surface opacity often observed with dilating drops).

## SIMILAR MOLECULAR PATHOGENIC SIGNALING PATHWAYS IN POAG TRABECULAR MESHWORK AND OPTIC NERVE HEAD CELLS AND TISSUES

**Abbot Clark**

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**Purpose:** The two sites of initial glaucoma damage to the POAG eye are the trabecular meshwork (TM), responsible for elevated intraocular pressure, and the lamina cribrosa (LC) within the optic nerve head (ONH), where unmyelinated RGC axons are first damaged. We have been using cells and tissues from POAG and age-matched control donor eyes to discover molecular pathogenic pathways in the TM and the ONH. Our purpose is to compare several of these molecular pathogenic signaling pathways in the glaucomatous TM and ONH as well as their effects on the extracellular matrix and cytoskeleton.

**Methods:** TM and ONH cells were cultured from POAG human donor eyes and age-matched control eyes. Anterior and posterior segments of POAG and age-matched control eyes were formalin fixed and paraffin embedded for immunostaining. RNA and protein expression were compared between glaucoma and age-matched control cells and tissues.

**Results:** Expression of the proinflammatory cytokine TGFb2 was significantly higher in GTM cells and GONH tissues. TGFb2 significantly increased expression of multiple ECM proteins in TM and ONH cells. GTM and GONH cells and tissues expressed higher levels of ECM proteins. BMPs 4 and 7 antagonize the profibrotic effects of TGFb2, but this protective effect is blocked by over-expression of the BMP antagonist Gremlin (GREM1) in both GTM and GONH cells and tissues. There is a dramatic rearrangement of the actin cytoskeleton to form CLANs in GTM cells and tissues and in GONH cells.

**Conclusion:** A similar profibrotic environment due to enhanced TGFb2/GREM1/signaling develops in glaucomatous TM and ONH cells and tissues, leading to enhanced ECM deposition. In addition, remodeling of the actin cytoskeleton further compromises signaling between these cells and their altered environment, continuing to exacerbate damage to the TM and ONH.

## ELECTROPHYSIOLOGIC RESPONSE TO PROLONGED MANOMETRIC IOP ELEVATION IN THE LIVING HUMAN EYE

**Mary Anne Garner[1], Mark Clark[2], Mitzi Swift[2], Ryan Strickland[1], Melody East[3], Alecia Gross[1], Massimo Fazio[2], Gregory Grossman[3], Alan Blake[3], Christopher Girkin4**

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We have previously evaluated the immediate biomechanical response of acute IOP elevation on the living human eye in research-consented brain-dead organ donors. To evaluate the cellular response of prolonged sub-ischemic IOP elevation on the human optic nerve and retina, we have developed methods to elevate IOP precisely over several hours, creating the first unilateral ocular hypertensive model in the living human eye. To determine if prolonged IOP exposure generates an isolated retinal ganglion cell (RGC) insult indicative of a sub-ischemic injury, we measured full field electroretinography (ERG) responses in 6 research-consented brain-dead organ donors using a red stimulus (3.4 Hz and 1.0 cd*s/m2) on a blue background (10 cd/m2) at baseline, immediate, 1 hour, 2 hours, 3 hours, and 4 hours after IOP elevation, and 1 hour after return to IOP baseline. Blood pressure (BP) was recorded at each timepoint, and ocular perfusion pressure (OPP) was calculated. Whole globes were then enucleated 5-12 hours following IOP elevation for ex vivo comparison of the molecular, transcriptomic, and cellular response between treated and control eye. A-wave, b-wave and photopic negative response (PhNR) amplitudes were compared across timepoints and correlated with changes in IOP, OPP, and BP. Our results show a selective decrease in the mean PhNR amplitude (mean -4.4 µV [4-hour exposure] vs. -6.89 µV [baseline]) averaged across donors, while the a-wave (-13 µV [baseline] vs. -13.02 µV [4-hour exposure]) and b-wave amplitudes (46.13 µV [baseline] vs. 44.96 µV [4-hour exposure]) were unchanged. Following the return to baseline IOP, the PhNR recovered (-6.9 µV [recovery] vs. -6.89 µV [baseline]). These results indicate that prolonged IOP elevation to 30 mmHg in the organ donor eye for 4 hours can selectively and reversibly inhibit RGC function while sparing outer retinal function.

## AUTOMATED QUANTIFICATION OF PERICYTE DENSITY ACROSS RETINAL CAPILLARY PLEXUSES IN 3D USING FLUORESCENT MOUSE MODELS

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Retinal pericyte dysfunction is increasingly recognized as a key feature of glaucomatous and neurovascular diseases, yet current quantification methods remain limited in standardization, dimensionality, and scalability. We previously developed a machine learning-based algorithm to perform automated three-dimensional segmentation and quantification of pericytes in PDGFRB-Cre TdTomato mice, where pericytes are specifically labeled and imaged using a confocal fluorescence microscopy. In this follow-up study, we applied the algorithm to quantify pericyte density in each of the three retinal vascular plexuses across 24 locations from three flat-mounted retinas. We first extracted the total number of pericytes within each high-resolution z-stack, then used a separate image processing algorithm to measure the total capillary length within the superficial, intermediate, and deep vascular plexuses. This enabled the calculation of three-dimensional capillary-normalized pericyte density across the retinal layers. Our results show pericyte densities of 33.6 ± 4.9 pericytes/mm in the superficial plexus, 27.4 ± 3.7 pericytes/mm in the intermediate plexus, and 25.4 ± 3.6 pericytes/mm of vasculature in the deep plexus. This platform allows for high-throughput, objective assessment of pericyte coverage and distribution in 3D across vascular compartments. We anticipate leveraging these quantitative tools to examine longitudinal changes in pericyte density and vascular integrity in animal models of disease, and ultimately in human subjects using advanced imaging modalities. These results represent an important step toward quantitative biomarkers of microvascular health with translational potential for early detection and monitoring of glaucoma.

## EFFECTS OF ORAL NICOTINAMIDE (NAM) OR NICOTINAMIDE RIBOSIDE (NR) IN THE DBA/2J MOUSE MODEL OF LATE ONSET, CHRONIC PIGMENTARY GLAUCOMA

**Jeffrey Boatright[1], Nan Zhang[2], Ying Li[3], Xian Zhang[4], Micah Chrenek[3], Jiaxing Wang[3], Preston Girardot[1], Jana Sellers[1], Xiangqin Cui[1], John Nickerson[3], Eldon Geisert3**

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We assessed the effects of oral administration of NAM or NR, precursors of nicotinamide adenine dinucleotide (NAD+), in DBA/2J mice. Administration of precursors at equivalent molar doses in food and/or water started at 4 or 9 months of age (MOA) and continued to 12 MOA (N = 36-45/cohort; NR: 1150mg/kg or 4200mg/kg; NAM: 500mg/kg or 2000mg/kg of body weight per day). Control cohorts identically received food/water without precursors. Intraocular pressure (IOP) was measured monthly. Retinal ganglion cell (RGC) function was assessed by pattern electroretinography (PERG) at 4, 6, 9, and 12 MOA. Iris pathology was assessed at 12 MOA by slit lamp transillumination. RGC density was assessed by Brn3a immunofluorescent confocal microscopy on fixed retinal whole mounts and axon numbers were quantified from optic nerve sections stained with toluidine blue. Retinal NAD+ tissues levels were enzymatically assayed. Administration of either precursor at high dose starting at 4 MOA partially but statistically significantly preserved retinal NAD+ levels, PERG amplitudes, axon densities, and iris morphology, all in dose-dependent manners. Administration of NR, but not NAM, preserved RGC density and delayed elevation of IOP. Administration of either precursor at high dose starting at 9 MOA partially preserved PERG amplitudes, with other outcomes trending towards protection. These data suggest that oral administration of NR or NAM protects against the multiple sequelae of the DBA/2J glaucoma mouse model. Of note, precursor treatment in ongoing disease was protective, and long-term prophylactic treatment with NR supplementation exhibited modestly greater potency and efficacy compared to that of NAM. Overall, these data support further exploration of NR supplementation as a glaucoma therapy.

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# PLATFORM SESSION 7
# GO WITH THE FLOW

## 4:00 PM - 5:15 PM

## EMORY AMPHITHEATER

## THE IMPACT OF THE GLAUCOMA-ASSOCIATED N700S VARIANT ON THE PROTEOLYTIC CLEAVAGE OF THROMBOSPONDIN-1

**Kate Keller[1], Ylng Ying Sun[1], Yong-feng Yang[1], Joanne Murphy-Ullrich[2], Paul Holden[1]**

*[1]Oregon Health & Science University, Portland, United States, [2]University of Alabama at Birmingham, Birmingham, United States*

The extracellular matrix molecule, thrombospondin-1 (TSP-1), is a component of the trabecular meshwork (TM). Previous studies showed that TSP-1 null mice have lower intraocular pressure (IOP), but the TSP-1 missense variant, *rs2228262* N700S, is associated with elevated IOP and primary open-angle glaucoma. The purpose of this study was to determine if the N700S variant impacts proteolytic cleavage of TSP-1. We genotyped glaucomatous TM cells and performed gene expression analysis on wild-type (n = 4) and heterozygous N700S (n = 5) cells using fluorescent barcode technology. Out of 31 differentially expressed genes, 4 proteases (MMP2, MMP16, CTSL, C1S) and 2 protease inhibitors (TIMP1, SERPING1) were up-regulated in N700S glaucoma TM cells. To investigate TSP-1 proteolytic cleavage in vitro, recombinant TSP-1 was over-expressed in HEK-293 cells and then digested with a panel of MMPs. Proteolytic fragments were identified using antibodies against the N-terminal domain or a C-terminal 6x histidine tag. These assays showed that TSP-1 is a novel substrate for MMPs -1, -7 and -10. In addition, ADAMTS1 and kallikrein-related peptidase-4 (KLK4) cleave the TSP-1 N-terminus. Next, we asked whether the N700S mutation affected proteolytic cleavage, which required generation of intact recombinant TSP-1 protein. To do this, CRISPR/Cas9 gene editing was used to knockout ADAMTS1 in 293 cells and a serine protease inhibitor was added to the culture media. Preliminary experiments using full-length wild-type and N700S recombinant proteins suggested that the N700S mutant may be folded differently than wild-type TSP-1, thereby affecting its proteolysis. In conclusion, the novel proteolytic activity of MMP-1 on TSP-1, and the up-regulation of its inhibitor, TIMP1, in N700S heterozygous glaucomatous TM cells, suggests that MMP1-mediated cleavage of TSP-1 is compromised in the N700S glaucomatous TM. Since TSP-1 mediates many cell-cell and cell-matrix interactions, the N700S variant may detrimentally affect matrix organization critical to IOP homeostasis in the TM.

## CGRP REGULATES THE FORMATION OF HIGH FLOW REGIONS IN THE TRABECULAR MESHWORK AND SCHLEMM'S CANAL

**Timur Mavlyutov, Colleen McDowell**
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The aqueous humor outflow pathway through the trabecular meshwork (TM) and Schlemm's canal (SC) has regions of high and low flow. The mechanism of how these regions are developed and regulated is unknown. CGRP positive C-fibers are the major type of sensory neurons innervating the TM/SC. Upon activation these neurons secrete CGRP locally, which then act on surrounding cells. Here, we determined whether CGRP positive neurites are involved in formation of high and low-flow regions. Flow regions were evaluated in 9-month-old C57BL/6J mice using fluorescent tracer beads (0.02 μm). Anterior segment flat mounts were co-labeled with antibodies against CGRP (afferent neurons), CALCRL (CGRP receptor), and PECAM1 (SC endothelium), and imaged by confocal microscopy to acquire tiled Z-stacks of entire outflow area. Density of CGRP neurites in each flow region was determined by volumetric analysis in ImageJ. Significantly more CGRP positive neurites were identified in high-flow regions compared to low-flow regions ($p < 0.05$, $n = 10$ eyes). CALCRL was shown to be expressed in TM and SC cells with significantly more expression in high-flow regions compared to low-flow regions ($p < 0.01$, $n = 5$ eyes). In situ hybridization detected expression of CGRP co-receptor RAMP1 in the TM with expression identified in cells closely located to CGRP positive neurites in this region. Primary human TM cells ($n = 3$ cells strains) were treated with or without CGRP (0.1 - 3 mM) for 72 hours and processed for western blot analysis, or exposed to fluorescently labeled beads with or without CGRP for 24 hours and analyzed for phagocytosis. CGRP treatment in primary TM cells resulted in increased phagocytosis ($p < 0.01$), decreased collagen1, fibronectin, and smooth muscle actin expression compared to control ($p < 0.05$), and attenuated TGFβ2 induced expression of these proteins ($p < 0.05$). These data suggest innervation of CGRP positive C-fibers influence the formation of high and low-flow regions in the TM and SC.

## WHAT OUTLIER MYOCILIN VARIANTS CAN TEACH US ABOUT PROTEOSTASIS DECLINE AND POTENTIAL NEW INTERVENTIONS FOR GLAUCOMA

**Raquel Lieberman[1], Kamisha Hill[1], Hannah Youngblood[1], Hailee Scelsi[1], Jennifer Faralli[2], Elijah Dunn[1], Tatsuo Itakura[3], M. Fini[4], Donna Peters[2]**

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Rare familial missense mutations in myocilin are associated with early-onset glaucoma due to a toxic gain of function caused by mutant protein misfolding. For most pathogenic myocilin mutants, an accompanying biophysical signature is mutant protein destabilization. However, there are outliers. For example, A427T, found in late-onset cases and unaffected family members, exhibits only marginal destabilization. Likewise, the prevalent premature stop Q368X does not contribute to early-onset glaucoma but increases overall risk for glaucoma. Here, we focus on the cellular and biophysical defects associated with these outliers. A427T and Q368X myocilin variants accumulate intracellularly when expressed in an inducible immortalized trabecular meshwork cell line. Inhibition of the proteasome reroutes wild-type myocilin, but not myocilin variants, from proteasomal degradation to lysosomal degradation. Whereas Q368X cannot be isolated for in vitro characterization, our structure of the A427T olfactomedin domain of myocilin shows modest perturbations largely confined to the mutation site. The examples of A427T and Q368X demonstrate that mutations that minimally or maximally perturb myocilin structure still present challenges for trabecular meshwork protein quality control clearance pathways. In the context of proteostasis decline with age, our study provides a rationale for how the accumulation of myocilin variants that are not causal for early onset glaucoma can contribute to glaucoma pathogenesis over time. More broadly, our study supports a therapeutic strategy aimed at enhancing autophagic clearance of accumulating proteins in the aging eye.

## ACTIVATION OF AVB3 INTEGRIN IS ASSOCIATED WITH A MYOFIBROBLAST PHENOTYPE IN TRABECULAR MESHWORK CELLS

**Donna Peters[1], Jennifer Faralli[1], Mark Filla[1], Kate Keller[2]**

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Like many other chronic age-related diseases, there is increasing evidence that fibrotic-like changes play a role in the pathogenesis of primary open-angle glaucoma (POAG). Our recent studies suggest that an age-related dysregulation of αvβ3 integrin activity may contribute to the profibrotic phenotype of human TM (HTM) cells observed in POAG. In this study, we investigated whether age-related activation of αvβ3 integrin is involved as an early event in initiating fibrotic-like changes in the TM that could restrict aqueous outflow. Using HTM cells isolated from young (< 40 yrs) and old (> 50 yrs) donor eyes, western blot and immunofluorescence microscopy studies showed that increased levels of αSMA expression observed in old TM cells correlated with higher levels of αvβ3 integrin activity. Expression of αSMA expression was dependent on αvβ3 integrin levels since shRNA knock down of αvβ3 integrin in old TM cells resulted in a statistically significant reduction in the levels of αSMA mRNA and protein levels. Knockdown of αvβ3 integrin also resulted in a reduction in the mRNA levels of classic fibrosis biomarkers, Snai2, VIM, and TWIST1, further suggesting that activation of αvβ3 integrin may influence expression of other profibrotic biomarkers. To determine if this was an age-related effect, we then maintained confluent cultures of young TM cells for 21 days in culture. As expected from our previous studies, western blot analysis indicated that young TM cells increased αSMA protein levels over time. Interestingly, we also saw an increase in αvβ3 integrin levels and by day 21, there was a robust increase in the formation of αSMA stress fibers that corresponded to TM cells expressing higher levels of activated αvβ3 integrin. These studies suggest that an age-related activation of αvβ3 integrin signaling represents an important early molecular event in inducing fibrogenic pathways associated with POAG.

## COMPARATIVE MULTIOMICS ANALYSIS OF THE IRIDOCORNEAL REGION OF DBA/2J AND DBA/2J-GPNMB$^{+}$/SJJ MICE

**Paloma Liton, Aleks Grimsrud, Vaibhav Desikan, Mi Sun Sung, Myoungsup Sim**
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Here, we present the first integrated transcriptomic and proteomic profiling of the iridocorneal region in DBA/2J mice and Gpnmb$^{+}$ controls to define molecular changes associated with ocular hypertension and glaucoma. Using RNA-seq and high-resolution, label-free quantitative proteomics, we identified over 20,000 transcripts and 8,500 proteins, yielding a molecular atlas of glaucoma-associated changes in DBA/2J mice. Principal component analysis and differential expression revealed clear genotype-specific signatures.

Gene ontology and Markov clustering of transcriptionally upregulated genes (> 1.5 fold, $p < 0.05$) highlighted enrichment in extracellular matrix (ECM) remodeling, collagen fibril organization, TGF-β signaling, and pro-inflammatory pathways in the angle region of DBA/2J mice compared to Gpnmb+. Proteomic analysis confirmed upregulation of complement components, antigen presentation machinery, and autophagy regulators. Integration of transcriptomic and proteomic data revealed 29 concordantly upregulated genes (> 1.5 fold, $p < 0.05$) - including *Col1a1*, *Fbln2*, *Ltbp2*, *Loxl1*, *Vcam1*, and *Serpine1* - which form functional clusters related to ECM structure, fibrinolysis inhibition, and complement activation. Genes downregulated in DBA/2J clustered around melanocyte differentiation, melanosome biogenesis, and vesicle exocytosis, consistent with the known roles of GPNMB in pigment-organelle maturation. Of interest is the reduced expression of *Pmel*, a gene linked to ocular pigment dispersion and human pigmentary glaucoma.

Differentially expressed genes and proteins were cross-referenced with human glaucoma and ocular hypertension risk loice identified by GWAS studies. Four overlapping genes were identified, including *Ltbp2*, *Loxl1*, *Col11a1*, and *Vcam1*, with upregulated expression at both the mRNA and protein levels in DBA/2J. *Angpt* and *Lmx1b* - linked to ocular hypertension - were transcriptionally downregulated.

Together, these findings support support the existence of an immune-fibrotic feed-forward loop in the pathology of DBA/2J mice and further support the concept at collagen–elastic-fiber pathology represents a core pathogenic mechanism in ocular hypertension and glaucoma.

# PLATFORM SESSION 8
# GLIAL CELLS: THE GOOD, THE BAD AND THE UGLY?

## 8:45 AM - 10:30 AM

## EMORY AMPHITHEATER

## MODELING NEUROINFLAMMATORY COMPONENTS OF GLAUCOMA IN VITRO WITH A TRIPLE CO-CULTURE OF HUMAN INDUCED PLURIPOTENT STEM CELL-DERIVED MICROGLIA, ASTROCYTES, AND RETINAL GANGLION CELLS

**Jade Harkin, Cátia Gomes, Kaylee Tutrow, Shruti Patil, Reham Afify, Jason Meyer**
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Glaucoma is the leading cause of irreversible blindness worldwide, affecting approximately 80 million people. Glial activation in this disease has been associated with morphological changes, increased proliferation, and the release of inflammatory mediators that contribute to the neurodegeneration of retinal ganglion cells (RGCs). However, there remains a critical need for human cellular models to investigate the crosstalk among microglia, astrocytes, and RGCs, and the role of these interactions in glaucomatous neurodegeneration. In this study, we differentiated astrocytes, microglia, and RGCs from human induced pluripotent stem cells (iPSCs). Microglial activation was induced using lipopolysaccharide (LPS) and confirmed through morphological assessment, immunostaining, and cytokine/chemokine profiling. LPS treatment significantly elevated microglial secretion of inflammatory cytokines, including IL-6, IL-8, IL-1β, and TNFα. Healthy and LPS-activated microglia were then co-cultured with RGCs, revealing that activated microglia reduced RGC neurite complexity and decreased neuronal excitability, as measured by multielectrode array. When co-cultured with astrocytes, LPS-activated microglia induced a reactive, pro-inflammatory astrocyte phenotype. Finally, in tri-culture systems of microglia, astrocytes, and RGCs, single-cell RNA sequencing revealed significant transcriptional changes in each cell type, indicating responses to their microenvironmental cues. Notably, RGC degeneration was more pronounced in the presence of both activated microglia and astrocytes, underscoring the synergistic impact of glial inflammation. Together, these findings establish a novel human iPSC-based model to study the cellular interactions among microglia, astrocytes, and RGCs, and demonstrate the utility of this system for probing the mechanisms of neuroinflammation and neurodegeneration relevant to glaucoma.

## THE CUMULATIVE ROLE OF REPEATED IOP ELEVATIONS IN EPIGENETIC TISSUE REPROGRAMMING AND INFLAMMATION

**Dorota Skowronska Krawczyk**
*University of California Irvine, Center for Translational Vision Research, Department of Physiology and Biophysics, Department of Ophthalmology, Irvine, United States*

Aging is a universal process that impacts all cells in an organism and is a significant risk factor for a range of neuropathies, including glaucoma. One of the key stresses contributing to glaucoma is elevated intraocular pressure (IOP). To better understand the molecular consequences of IOP challenges on vision and retinal ganglion cell (RGC) health, we developed a new mouse model. Our findings reveal that susceptibility to tissue degeneration can be detected before RGC death occurs and is first observable at the chromatin level. Additionally, we show how repeated instances of IOP elevation reprogram the tissue, leading to accelerated aging in the retina of young individuals. Finally, we propose the use of specific anti-inflammatory molecules as a potential strategy to slow the degeneration of RGCs caused by IOP elevation. These results may help explain the challenges in managing glaucomatous insults, such as elevated IOP, in older individuals and suggest novel therapeutic approaches for managing age-related eye diseases, including glaucoma.

## RESIDENT TISSUE MACROPHAGES GOVERN INTRAOCULAR PRESSURE HOMEOSTASIS

**Katy Liu[1], Maria Fernanda Suarez[1], Aleks Grimsrud[1], Darren Schuman[1], Revathi Balasubramanian[2], Simon John[2], W. Daniel Stamer[1], Daniel Saban[1]**

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Intraocular pressure (IOP) is tightly regulated by the conventional outflow tissues, preventing ocular hypertension that leads to neurodegeneration of the optic nerve, or glaucoma. Although macrophages reside throughout the conventional outflow tract, their role in regulating intraocular pressure remains unknown. Their developmental lineage, or ontogeny, plays an important role in macrophage phenotype and functionality. “Resident” tissue macrophages (RTMs) are derived prenatally and are long-lived, whereas monocyte derived macrophages are adult bone marrow derived and short-lived. In this study, we determine the distribution and function of RTMs in IOP homeostasis.

Using *Cx3cr1-YFP*$^{CreER/+}$ mice with various Cre reporters, RTMs were conditionally labeled by tamoxifen pulse administration and subsequent wash out. RTMs were depleted by targeting Cre driven iDTR (inducible diptheria toxin receptor). Intraocular pressure (IOP) was measured by tonometry and outflow facility was measured using the iPerfusion system. Anterior segment whole mounts were fixed and labeled, and macrophages were identified as YFP+ (Cx3cr1+) cells by confocal microscopy. Student’s t test with $p < 0.05$ was considered significant.

We uncovered a dual macrophage ontogeny with distinct spatial organizations across the mouse lifespan. RTMs concentrated in the trabecular meshwork and Schlemm’s canal, whereas short-lived monocyte-derived macrophages, instead, were abundant around distal vessels. Specific depletion of RTMs triggered elevated IOP (18.4 ± 0.4 mmHg versus 16.9 ± 0.3 mmHg, $p < 0.0002$) and reduced outflow resistance eyes (1.0 ± 0.1 versus 1.7 ± 0.2 nl/min/mmHg, $p < 0.002$), linked to aberrant extracellular matrix turnover in the resistance-generating tissues of the trabecular meshwork. This dysregulated physiology and tissue remodeling were not observed when we depleted monocyte-derived macrophages.

Our study shows ontogeny and tissue-specific macrophage function within the outflow tract, uncovering the integral homeostatic role of RTMs in resistance-generating tissues whose dysfunction is responsible for glaucoma.

## ASTROCYTE POLARIZATION AND POLARIZED MICROGLIAL ACTIVATION

**Claire H. Mitchell, Keith Campagno, Wennan Lu**
*Philadelphia, United States*

Microglia and astrocytes contribute to glaucoma pathophysiology, but signals connecting IOP elevation to glial responses are unclear. As IOP elevation triggers a mechanosensitive release of ATP, we asked if the P2X7 receptor (P2X7R) for ATP contributed to astrocyte polarization or the IL-1β response. IOP elevation increased *C3*, *Serping1*, and *H2-T23*, associated with the A1 astrocyte state, and pan-astrocyte markers *Gfap*, *Steap4*, and *Vim*. *Tnfa*, *IL1a*, and *C1qa*, associated with microglial activation, also increased. This response was missing in P2X7KO mice. Intravitreal injection of P2X7R agonist BzATP in normotensive eyes also increased these activation markers, suggesting the receptor was sufficient for astrocyte polarization. Correlation analysis indicated a complex association between genes. As P2X7R activates the NLRP3 inflammasome and IL-1β maturation, protein levels were examined. While P2X7R stimulation increased IL-1β throughout the retina, the microglial response was primarily in the outer retina, while the inner retinal rise was predominantly in the ganglion cell layer. In summary, the P2X7R is necessary and sufficient for activation of A1 astrocytes and associated microglial markers after IOP elevation. The rise of IL-1β in outer retinal microglia, and the impact of the IL-1β rise in the ganglion cell layer, remain to be determined.

# MICROGLIAL ACTIVATION AND SENESCENCE-ASSOCIATED SECRETORY PHENOTYPE IN RESPONSE TO ACUTE IOP INCREASE IN LIVING HUMAN MACULA

**McKenna Somerville[1], Ryan Strickland[1], Sydney Lin[1], Mary Anne Garner[1], Massimo Fazio[2], Christopher Girkin[3], Alecia Gross[1]**

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Elevated intraocular pressure (IOP) is a primary risk factor for glaucoma. Animal models of acute IOP elevation have demonstrated immune responses that include microglial activation and senescence associated secretory phenotype (SASP) response. This study evaluates changes in microglial reactivity and SASP responses spatially across the macula following acute unilateral IOP elevation in the living human eye of brain-dead organ donors.

One randomly selected eye from three research-consented brain-dead organ donors underwent IOP elevation achieved by injection of viscoelastic gel into the anterior chamber. This injection resulted in a sustained IOP elevation (mean follow-up IOP: 41.46 mmHg) for 8-9 hours until organ procurement. IOP was measured using rebound tonometry every 2 hours until organ procurement, after which ocular tissues were enucleated, dissected, formalin-fixed, and paraffin embedded. 5 µm sections of macula were used for immunohistochemistry, RNA fluorescent *in-situ* hybridization and spatial transcriptomics. Subsequent analysis was performed across 6 parafoveal regions and in the fovea to elucidate differential cellular response.

In our initial two donors, immunohistochemistry showed greater IBA1-positive microglia surface area ($\Delta$ = 7.724 ± 4.2 µm$^2$) in the inner nuclear layer of the superior fovea of treated eyes. We found differential expression (represented as puncta per nuclei) for transcripts encoding the SASP transcripts *IL6* ($\Delta$ = -3.52 ± 0.52), *FASR* ($\Delta$ = -1.01 ± 0.52), *FGF2* ($\Delta$ = 0.89 ± 0.21), and *CCL5* ($\Delta$ = 1.66 ± 0.44). Preliminary findings from spatial transcriptomics revealed high prevalence of transcripts involved in IL6-signaling, SASP, MHC I/II presentation, oxidative-stress-induced senescence, toll-like receptor cascades and other immune markers.

These results suggest that, as with animal models, acute IOP in the human retina elicits both microglial and SASP responses. Understanding these molecular responses in the human, which have been shown to increase the susceptibility of retinal ganglion cells to injury, may elucidate conserved pathways across species that could be evaluated to reduce the impact of these injurious cascades on IOP-related retinal injury.

## COMPLEMENT C3 ACTIVATION IN REACTIVE ASTROCYTES DRIVES AXONAL DEGENERATION OF RETINAL GANGLION CELLS IN HUMAN PLURIPOTENT STEM CELL-DERIVED MODELS

**Cátia Gomes, Kang-Chieh Huang, Jade Harkin, Carson Prosser, Aaron Baker, Laurna Varghese, Parker Wilcox, Tsai-Yu Lin, Jason Meyer**

*Indiana University School of Medicine, Indianapolis, IN, United States*

Astrocytes support retinal ganglion cells (RGCs) in the retinal nerve fiber layer and optic nerve but may also contribute to their degeneration during the course of glaucoma pathology. Complement cascade activation is a hallmark characteristic of glaucoma, with neurotoxic reactive astrocytes expressing elevated complement C3 levels. However, the mechanisms linking astrocyte neurotoxicity and complement activation remain unclear. To investigate the role of reactive astrocytes in RGC degeneration and the contribution of the complement cascade, both RGCs and astrocytes were differentiated from human pluripotent stem cells (hPSCs). Microfluidic platforms were developed to replicate the compartmentalized nature of RGCs and to properly position astrocytes along the proximal axonal compartment, mirroring the location of reactive astrocytes in the optic nerve head. A reactive phenotype was induced in hPSC-derived astrocytes using C1q, TNFα, and IL1α, and shRNA techniques were employed to modulate complement C3 expression in astrocytes. Following induction of a reactive state, hPSC-derived astrocytes displayed a marked hypertrophic profile, upregulation of inflammatory genes, and increased cytokine secretion. Using microfluidic platforms, we observed that reactive astrocytes exerted degenerative effects upon RGCs when uniquely placed along the proximal axonal compartment, spatially similar to the region affected in the optic nerve head. The downregulation of complement C3 expression via shRNA resulted in a less pronounced hypertrophic profile, decreased concentration of soluble toxic factors and overall attenuated reactivity-associated features in astrocytes. Notably, targeting complement C3 in astrocytes located along the proximal axonal region of RGCs decreased axonal degenerative phenotypes, indicating that complement activation plays a key role in driving both astrocyte reactivity and subsequent RGC axonal degeneration. Taken together, these results demonstrate that reactive astrocytes contribute to RGC axonal degeneration and suggest that targeting the complement cascade in reactive astrocytes may represent a promising therapeutic approach for addressing the neuroinflammatory components of glaucoma.

## A TRANSCRIPTOMIC ATLAS OF IMMUNE CELL POPULATIONS IN GLAUCOMATOUS MOUSE RETINA AND OPTIC NERVE

**Zhuoran Yin[1], James Harris[1], Paul Cullen[1], Yixi Xue[1], Anthony Mukwaya[1], Dario Tommasini[2], George Baldwin[1], Nasir Uddin[1], Navita Lopez[3], Daniel Sun[1], Sandro Da Mesquita[4], Monica Vetter[3], Karthik Shekhar[2], Milica Margeta[1]**

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Immune cells play a critical role in pathogenesis of glaucoma. However, their ontogeny, gene expression profiles, spatial distribution, and functional changes in the retina and optic nerve in glaucoma remain poorly understood. We performed single-cell RNA sequencing to investigate changes in immune cell populations in the microbead mouse model of glaucoma. Elevated intraocular pressure was induced by injecting microbeads into the anterior chamber of 3-month-old C57BL/6J mice, while control mice received sham injections. Retinas and optic nerves were harvested 30 days after injection, and $CD45^+$ immune cells were isolated using flow cytometry and sequenced using 10X Genomics. A total of 28,277 immune cells were successfully sequenced from microbead- and sham-injected retinas and optic nerves, representing a range of immune cell types. In control animals, we found a unique myeloid cell population in the optic nerve head (ONH), characterized by low expression levels of the classic homeostatic microglial marker P2ry12; notably, we have also detected this population in the human ONH. Furthermore, ONH myeloid cells also express MHCII, CD11c (Itgax), and CD25 (Il2ra), suggesting a critical role for immune activation and antigen presentation in the ONH in homeostasis. In disease, we noted dramatic changes in glaucomatous retinas, while changes in the retrolaminar optic nerves from the same animals were unexpectedly subtle. Retinas from mice with experimental glaucoma demonstrated significant increases in disease-associated microglia, activated (ICOS+) T cells, and MHCII+ macrophages compared to sham-injected retinas. Interestingly, MHCII+ macrophages in the glaucomatous retina were perivascular in location and partially derived from infiltrating monocytes as determined by lineage tracing. Taken together, these findings provide novel insights into the immune cell landscape in glaucoma, suggest crosstalk between retinal microglia, macrophages and peripheral immune cells, and may serve to identify new therapeutic targets for this common blinding disease.

# PLATFORM SESSION 9
# EYE AM UNDER PRESSURE :-(

## 11:00 AM - 12:00 PM

## EMORY AMPHITHEATER

## QLS-111-FDC, A FIXED DOSE COMBINATION OF QLS-111 WITH LATANOPROST, EFFECTIVELY LOWERS IOP IN A NORMOTENSIVE PRECLINCAL MOUSE MODEL

**Mike Fautsch[1], Uttio Roy Chowdhury[2], Lisa Brandano[2], Ralph Casale[2], Daniel Dewalt[2], Laura Rykard[2], Thurein Htoo[2], Barbara Wirostko[2]**

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Current glaucoma therapies reduce intraocular pressure (IOP) by decreasing aqueous humor production or enhancing uveoscleral and trabecular outflow, but none directly target episcleral venous pressure (EVP), which accounts for up to 60% of IOP and sets the physiological floor for IOP lowering. Qlaris Bio has developed QLS-111, a novel ATP sensitive potassium channel opener formulation that offers enhanced stability and bioavailability of drug substance and lowers IOP by specifically reducing EVP. In a phase 2 trial (Apteryx, NCT06249152), QLS-111 (0.015%, QPM) achieved an additional 3.2 mmHg IOP reduction over latanoprost in ocular hypertension and/or primary open angle glaucoma patients, from a baseline of 19.8 mmHg. The present study assessed the safety and IOP-lowering efficacy of QLS-111-FDC, a fixed dose combination of QLS-111 (0.015%) and latanoprost (0.005%) in normotensive C57BL/6J mice. Mice (n = 10) were divided into two groups and baseline IOP measurements were obtained with a rebound tonometer. One eye was treated with either a 5 µl bolus of QLS-111 (0.015%, n = 5) or latanoprost (0.005%, n = 5) once daily for 4 days, while the fellow eye received vehicle. On days 5–8, the treated eye received QLS-111-FDC once daily. Baseline IOPs were similar between groups (16.38 ± 0.52 mmHg vs.16.33 ± 0.21 mmHg). Monotherapy with QLS-111 and latanoprost lowered IOP by 4.53 ± 0.28 mmHg (-27.71 ± 1.94%) and 4.09 ± 0.22 mmHg (-25.03 ± 1.17%) compared to baseline ($p < 0.0001$). Following QLS-111-FDC treatment, IOP was lowered by 6.52 mmHg ($p = 0.002$) and 5.89 mmHg ($p = 0.003$). Treatment with QLS-111-FDC reduced IOP to approximately 10 mmHg, a 40% change from baseline. No adverse events were observed during treatment. These results support QLS-111-FDC as a well-tolerated ocular hypotensive agent, offering enhanced IOP reduction compared to monotherapies with QLS-111 or latanoprost. Current data underscores the potential of QLS111-FDC as a promising candidate for further clinical development.

# ESTROGEN RECEPTOR SIGNALING AGAINST TGFB2-INDUCED TRANSCRIPTOMICS CHANGES ON HUMAN TRABECULAR MESHWORK CELLS

**Yutao Liu[1], Ola Elsayed[1], Jingwen Cai[1], Hongfang Yu[1], Hongyan Xu[2], Louis Pasquale[3], W. Daniel Stamer[4]**

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Estrogen signaling through ESR1, ESR2, and GPER1 receptors, has been implicated in modulating intraocular pressure (IOP). TGFβ2, a glaucoma-causing molecule, increases outflow resistance in the trabecular meshwork (TM) by altering cell contractility and extracellular matrix homeostasis, leading to increased IOP. We hypothesize that estrogen treatment can reverse the changes caused by TGFβ2 in TM cells. First, primary HTM cells from non-glaucomatous donors (6 females and 4 males) were treated with DMSO, TGFβ2 (10ng/μl), or TGFβ2 + estradiol (E2, 50 μM) under CMS (15%, 1 cycle/s) for 24 hours. Stranded total RNA-Seq and miRNA-Seq analysis using Partek-Flow were performed to identify differentially expressed (DE) genes and miRNAs. A set of 327 significant DE genes were identified after TGFβ2 treatment (FDR≤0.05, |FC| ≥ 2) and 285 of them became non-significant after the combined E2 treatment. These reversed genes were enriched in growth factor receptor binding and cell adhesion molecule binding. A set of 65 DE miRNAs were identified after TGFβ2 treatment (p≤0.05), and 54 of them became non-significant in the combined E2 treatment. Our miRNA-mRNA interaction analysis identified 58 of these 285 genes could be targeted by 28 DE miRNAs using IPA. We then treated HTM cells (6 females/4 males) with: DMSO, 10ng/ml TGFβ2 only, 10ng/ml TGFβ2 in presence of either: 50 μM PPT (ESR1 agonist), 50 μM DPN (ESR2 agonist), 50 μM G1 (GPER1 agonist), 50 μM G36 (GPER1 antagonist) under CMS for 6, 24, 48, and 72 hours, followed by gene expression profiling using ddPCR. Our linear mixed effect model-based statistical analysis indicated the significant impact of sex, time, and treatment in affecting GPER1 expression. Compared to 6-hour treatment, TGFβ2 reduced GPER1 expression significantly at 24hr followed by a significant increase at 48 and 72 hours. Compared to DMSO at 24-hour, TGFβ2 significantly increased ACTA2 expression, which was reversed by estrogen treatments. Females showed higher ACTA2 expression when compared to males. INHBA was significantly increased in 70% of the TM samples with TGFβ2 treatment and reversed by estrogen treatments. Our study indicated the anti-TGFβ2 impact of estrogen receptor signaling in HTM cells, suggesting its role in modulating IOP.

## ANGIOPOIETIN-LIKE PROTEIN 4 (ANGPTL4) CONTRIBUTES TO GLAUCOMATOUS CHANGES IN THE TRABECULAR MESHWORK AND OCULAR HYPERTENSION

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Overactivation of TGFβ signaling by elevated TGFβ2 contributes to glaucomatous ocular hypertensin (OHT), the most important risk factor and treatment target of primary open angle glaucoma (POAG). We have previously shown that activation of the canonical Wnt signaling antagonizes TGFβ2-induced changes in the trabecular meshwork (TM) and OHT. Using RNAseq, we discovered that angiopoietin-like protein 4 (ANGPTL4) was significantly upregulated by TGFβ signaling and this upregulation was inhibited by Wnt signaling in primary human TM (pHTM) cells. This finding led us to the hypothesis that ANGPTL4 contributes to glaucomatous changes in the TM as well as induces OHT. To test our hypothesis, we first compared the level of ANGPTL4 in POAG vs. non-POAG aqueous humor (AH) using ELISA. We found that ANGPTL4 was significantly elevated in the glaucomatous aqueous humor. Then, we overexpressed or knocked down ANGPTL4 in both transformed or primary HTM cells using a plasmid/lentivirus or siRNA, respectively. The overexpression of ANGPTL4 induced extracellular matrix (ECM) proteins, while the knock down of ANGPTL4 inhibited ECM. To determine if ANGPTL4 affects intraocular pressure (IOP), we perfused 3 pairs of human corneas with or without 2 ug/ml ANGPTL4 recombinant protein. ANGPTL4 elevated intraocular pressure (IOP) by more than ~8 mmHg from baseline in all 3 treated eyes, while there was no change in IOP in the fellow control eyes. Our perfusion cultured human cornea data suggest that ANGPTL4 affects the TM outflow pathway tissue. In summary, we believed that ANGPTL4 contributes to glaucomatous IOP elevation and its role in POAG OHT needs further investigation.

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## A ROLE FOR THE CATIONIC AMINO ACID TRANSPORTER SLC7A1 IN REGULATION OF AQUEOUS HUMOR OUTFLOW

**Manvi Goel[1], Graham Clark[2], Reynolds Ablordeppey[3], Fiona McDonell[3], Krishnakumar Kizhatil[1]**

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Schlemm's canal endothelial cells (SCE) form the final barrier to aqueous humor outflow and respond to elevated intraocular pressure (IOP) by producing nitric oxide (NO), which increases canal permeability. NO synthesis by endothelial NOS3 (eNOS) requires extracellular arginine despite adequate intracellular levels—a phenomenon known as the "arginine paradox." One proposed resolution is the spatial colocalization of NOS3 with the arginine transporter SLC7A1 within caveolae.

We investigated the role of SLC7A1 in regulating aqueous humor outflow. Using Prox1-GFP mice, we confirmed SLC7A1 localization in Schlemm's canal. Proximity ligation assays revealed SLC7A1 is associated with NOS3 and partially colocalized with the caveolar protein CAV1. We have also confirmed the localization of a population of SLC7A1 in caveolae using immunoelectron microscopy. To test functional relevance, we inhibited SLC7A1 using lysine in both human eyes and wild-type mice. Outflow facility decreased by 21% in lysine-treated eyes (3.14 ± 0.78 nl/min/mmHg) compared to controls (3.99 ± 0.75 nl/min/mmHg; $p = 0.003$), indicating reduced aqueous humor outflow. This effect was pressure-dependent and abolished in CAV1-null mice, implicating caveolae in the mechanism. Lysine also reduced outflow in human eyes.

Our findings suggest that SLC7A1 plays a regulatory role in aqueous humor outflow, likely by ensuring efficient NO production through close spatial association with NOS3 in caveolae—providing a molecular explanation for the arginine paradox. Ongoing work includes examining how caveolae loss affects NOS3-SLC7A1 interactions and using CRISPR to knock out SLC7A1 in Schlemm's canal endothelial cells to further define its functional role.

## CONDITIONAL DELETION OF AP-2B IN THE ANTERIOR SEGMENT OF ADULT MICE RESULTS IN ELEVATED IOP

**Judith West-Mays, Aysha Jamal, Lindsey Kukoly, Aftab Taiyab**
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Previously, our lab has demonstrated that deletion of AP-2β from the developing periocular mesenchyme (POM) of mice results in abnormal development of anterior angle structures including an absent TM and underdeveloped Schlemm's canal. To determine the role of AP-2β in the adult TM we have recently developed a new inducible mouse model by deleting AP-2β postnatally using a tamoxifen-inducible ubiquitin cre recombinase (Ubc-Cre-ERT2) system. *UbCre*$^{ERT2+/-}$; *Tfap2b*$^{+/-}$ mice were bred with *Tfap2b*$^{lox/lox}$; *tdTomato*$^{lox/lox}$ mice to generate pups containing *UbCre*$^{ERT2+/-}$; *Tfap2b*$^{-/lox}$; *tdTomato*$^{lox/+}$ (mutant) and *UbCre*$^{ERT2+/-}$; *Tfap2b*$^{+/lox}$; *tdTomato*$^{lox/+}$ (control). Tamoxifen was administered intraperitoneally to induce postnatal deletion of AP-2β. A total of four-day consecutive injections were administered from P22 to P25, with tamoxifen (Sigma) dissolved in corn oil and used at a dosage of 75 mg/kg body weight (3.75 µL per gram of body weight). IOP levels were measured in both UbCre/AP-2β-KO mice and control littermates at P40 and P60 using tonolab rebound tonometer (Topcon Canada, Inc.) with at least six readings per eye (mmHg). At P60 mice were euthanized, eyes were enucleated, fixed and processed for paraffin sectioning. H&E and immunohistochemistry (IHC) using antibodies for AP-2β and the TM marker such as αSMA were performed on paraffin sections from mutant and control eyes. Histological analysis of the UbCre/AP-2β KO mutants confirmed the presence of the TM and an open anterior chamber angle, similar to control littermates. However, a substantial loss of AP-2β and a-SMA expression was observed in the mutant TM region compared to controls. These changes were correlated with significantly elevated IOP observed at both P40 and P60, indicating a relationship between absence of AP-2β and impaired aqueous humor outflow in UbCre/AP-2β KO mutants. These findings suggest a role for AP-2b in maintenance of the TM, which is being further explored.

Supported by: NIH EY025789

# POSTER SESSION 1
# HANGING OUT WITH BRILLIANT IDEAS

## POSTER 01

# HOMEOSTATIC PLASTICITY IN THE DORSOLATERAL GENICULATE NUCLEUS OF DBA/2J MICE

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DBA/2J mice are a widely-used model of glaucoma characterized by an age-dependent increase in intraocular pressure (IOP). This leads to retinal ganglion cell (RGC) degeneration, optic nerve atrophy, and loss of retinal output synapses in the dorsolateral geniculate nucleus (dLGN) of the thalamus, a critical RGC projection target for visual signals en route to the primary visual cortex. The goal of this study was to determine whether and how elevated IOP triggers homeostatic adaptations in the population of excitatory neurons in the dLGN (thalamocortical/TC relay neurons). In our colony, DBA/2J mice, aged 11-13 months, had reduced pattern electroretinogram (PERG) responses that correlated with intraocular pressure and glial scarring in optic nerve cross sections. Using brain slice patch clamp recordings, we found that mice showed a reduction in the strength of retinal ganglion cell synaptic inputs to TC neurons. Despite this, TC neurons were able to support robust action potential output in response to optic tract stimulation, implying that they more efficiently transformed weakened synaptic inputs to action potential output. Probing the mechanisms underlying this, we found that TC neurons displayed enhanced intrinsic excitability, with lower action potential thresholds and lower rheobase. TC neurons had more variable input resistance and resting membrane potential and significantly reduced membrane capacitance. There was no change in the length of TC neuron axon initial segments, measured using ankyrin-G staining of histological dLGN sections. Together, these results indicate that dLGN TC neurons might be able to support normal signaling to the visual cortex even as high IOP leads to loss of signaling from the retina and that this is partly attributable to enhanced intrinsic excitability.

## POSTER 02

# DNAJC3 ENHANCES AXON REGENERATION IN A MOUSE MODEL OF OPTIC INJURY

**Eldon Geisert[1], Ying Li[2], Sandra Jardines[2], Su-Ting Lin[2], Fangyu Lin[2], Jiaxing Wang[2]**

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In Glaucoma retinal ganglion cell (RGC) death leads to a severe vision loss. Knocking down Pten or inducing an inflammatory response in the retina promotes RGC survival and axon regeneration. To identify genomic elements modulating this axon regeneration, we have taken a forward genetics approach using the BXD recombinant mouse strains. Axon regeneration was induced by knocking down Pten in RGCs using adeno-associated virus (AAV) to deliver an shRNA followed by an intravitreal injection of Zymosan with CPT-cAMP that produced a mild inflammatory response. Twelve days after optic nerve crush (ONC), regenerating axons were labeled by intravitreal injection of Cholera Toxin B (CTB) conjugated with Alexa Fluor 647. The number of regenerating axons and the distance they traveled were quantified. Across all 33 BXD strains examined there was a 7.5-fold difference in the number of regenerating axons and a 4-fold difference in the distance extended down the optic nerve. These phenotypic differences were used to generate an interval map defining genomic loci modulating the enhanced axonal regeneration. A quantitative trait locus modulating axon regeneration was identified on Chromosome 14 (115 to 119 Mb). Of the 16 annotated genes within this locus, Dnajc3, modulates axonal regeneration. *Dnajc3* encodes Heat Shock Protein 40 (HSP40), which is a molecular chaperone. Knocking down *Dnajc3* in the high regenerative strain (BXD90) led to a decreased regeneration response; while, overexpression of *Dnajc3* in a low regenerative strain (BXD34) resulted in an increased regeneration response. These findings reveal that *Dnajc3* not only increases the number of regenerating axons, it also increases the distance those axons travel. The enhanced regeneration will prove to be critical for functional recovery in humans, where the distance axons travel to their target is considerably longer than that of the mouse.

## POSTER 03

# LOSS- AND GAIN-OF-FUNCTION ESTABLISHES A CRITICAL PROTECTIVE ROLE FOR LIPOXINS IN OPTIC NERVE DEGENERATION AND NEUROINFLAMMATION

**Shubham Maurya[1], Branson Bajoua[1], Matangi Kumar[2], Cici Liu[1], Paripoorna Sharma[2], Jiwoo Han[1], Amodini Chaudhary[1], Sydney Adcook[1], Shruthi Karnam[1], John Flanagan[2], Karsten Gronert[2]**

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Lipoxin (LX) B4 is a homeostatic lipid mediator produced by macroglia. LXB4 treatment protects against retinal ganglion cell (RGC) degeneration during ocular hypertension (OHT) by regulating optic nerve (ON) microglia and preventing their polarization to a $CD74^+$ disease-associated microglia (DAM) phenotype. Using a loss- or gain-of-function approach, we investigated how endogenous lipoxin production impacts RGC degeneration and ON neuroinflammation by targeting 15-lipoxygenase (15-LOX), a key enzyme in lipoxin biosynthesis. A macroglia-specific conditional 15-LOX knockout ($GFAP^{cre}$; $Alox15^{cko}$) was generated as a loss-of-function model and investigated using a silicone oil model of OHT. Macroglia-specific deletion of 15-LOX accelerated ganglion cell complex (GCC) degeneration by 119%. MorphOMICs and pseudotime trajectory analyses of ON microglia morphology revealed a distinct phenotype shift associated with the lipoxin loss, including a 30% increase in $CD74^+$ DAMs. Bulk RNA sequencing of the ON, followed by pathway enrichment and NicheNet ligand-receptor analysis, showed that loss of 15-LOX led to a marked upregulation of microglia sensome markers (70%) and enrichment of immune response pathways. This increase in microglial inflammatory reactivity was associated with disrupted astrocyte and oligodendrocyte signaling to microglia and enhanced microglia-microglia interactions. Gain-of-function was achieved via retinal gene therapy by delivering a macroglia-specific AAV-human 15-LOX construct. 15-LOX gene therapy significantly protected against OHT, reducing GCC degeneration by 77% and ON axon loss by 65%. Therapeutic amplification of lipoxin signaling in retina restored the homeostatic phenotype of ON microglia, as assessed by morphOMICs and pseudotime trajectory analyses, and reduced $CD74^+$ DAM polarization by 55%. These findings demonstrate that the endogenous lipoxin pathway is a key regulator of ON neuroinflammation and polarization to the ON-specific $CD74^+$ DAM phenotype in response to OHT. Therapeutic activation of the lipoxin pathway via 15-LOX gene therapy is neuroprotective and highlights the importance of maintaining or restoring homeostatic microglia function in the ON.

## POSTER 04

# SINGLE-NUCLEI RNA SEQUENCING OF HUMAN TRABECULAR MESHWORK CELLS: EXPLORING CELLULAR DIVERSITY AND MOLECULAR MECHANISMS IN GLAUCOMA

**Prakadeeswari Gopala Krishnan[1], Pei Tan[2], Zaid Ahmed[1], Jinjing Jian[1], Mai-Linh Ton[1], Linya Li[1], Yogapriya Sundaresan[1], Balasankara Kaipa[1], Xiangmin Xu[3], Rui Chen[1], Gulab Zode[1]**

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The trabecular meshwork (TM) is a sieve-like, avascular connective tissue essential for regulating aqueous humor outflow resistance and intraocular pressure (IOP) and its dysfunction impairs outflow, causing elevated IOP and vision loss. Although glaucomatous damage likely alters TM cellular composition and molecular profiles, these changes remain poorly characterized. We used single-nucleus RNA sequencing (snRNA-seq) to create a detailed cellular atlas of human glaucomatous and healthy TM tissue, aiming to identify disease-associated cell types and gene expression changes linked to fibrosis and IOP regulation. Human TM tissues from 8 healthy and glaucomatous donors were collected within 0–10 hours postmortem. After blunt dissection and snap freezing, nuclei were isolated and sequenced using the 10x Genomics Chromium 3' v4 platform. After stringent quality control, a high-quality total of 256,192 nuclei from control and 303,648 nuclei from glaucomatous TM were retained for downstream analyses which were analyzed using scVI, Scanpy, anndata, and MILO frameworks. Cell type identification and differential gene expression (DE) analyses used thresholds of log fold change > 0.5 and $p < 0.05$. Pathway enrichment was performed with clusterProfiler in R. Sixteen TM cell populations were identified and found 440 genes differentially expressed in glaucomatous TM. Notable changes in TM cells involved extracellular matrix organization, cytoskeletal remodeling, and calcium signaling. Pathways related to ER stress, unfolded protein response, mitochondrial dysfunction, impaired autophagy, and TNF-α inflammation were activated. MILO analysis revealed a fibroblast-to-myofibroblast transition, indicating altered cell states in glaucoma. This study offers a comprehensive single-nucleus transcriptomic landscape of human TM, uncovering cellular diversity and pathological shifts associated with glaucoma. The identification of disease-enriched gene signatures and signaling disruptions, including calcium signaling and fibrosis-related transitions, provides critical insight into TM dysfunction and IOP dysregulation. These findings not only deepen our understanding of glaucoma pathobiology but also present novel targets for therapeutic intervention.

## POSTER 05

# COMPARATIVE OCULAR PARAMETERS OF THE ESR1 CONDITIONAL KNOCKOUT MOUSE

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Glaucoma risk may be influenced by sex-specific factors, including estrogen signaling, which has been linked to intraocular pressure (IOP) regulation and disease progression. Prior work shows that estrogen deficiency exacerbates vision loss after ocular injury, yet the direct role of estrogen in the retina remains unclear. This study aims to develop a retina-specific estrogen receptor 1 (ESR1) conditional knockout (ESR1cKO) mouse model using the Rx-Cre system to selectively disrupt ESR1 signaling in retinal progenitor cells. This model will enable future studies on the role of retinal ESR1 in glaucomatous injury, independent of systemic estrogen effects. For this study, ESR1cKO mice (male; n = 3–5, female; n = 4) and ESR1floxed mice (male; n = 6, female; n = 4–5) were bred in-house on a C57BL/6 background. For comparison, C57BL/6 male and female mice (n = 4 per sex) were purchased from Jackson Laboratories. We examined IOP using rebound tonometry and assessed spatial frequency, retinal structure, and retinal function by optomotor response (OMR), optical coherence tomography (OCT), and electroretinogram (ERG), respectively. No significant sex differences were found, so data were pooled for analysis. C57BL/6J mice had significantly higher IOP than ESR1floxed ($p = 0.0072$) and ESR1cKO ($p = 0.036$) groups. No significant differences were found in spatial frequency ($p = 0.51$) or retinal structure ($p = 0.19$). However, ESR1cKO mice showed significantly altered dark-adapted c-wave implicit time, light-adapted b-wave amplitude, and oscillatory potential amplitude compared to ESR1floxed and C57BL/6J cohorts—changes linked to retinal progenitor cell function. Future studies will increase sample size, include a Cre-only strain, and assess selective knockout of other estrogen receptors (ESR2, GPER). The ESR1cKO strain is a promising model for studying ESR1's neuroprotective potential in RGC injury and could inform future glaucoma therapies.

## POSTER 06

# ENHANCED AXON REGENERATION IN THE BXD29-TLR4LPS-2J/J MOUSE IS NOT DUE TO TLR4 MUTATION

**Fangyu Lin, Jiaxing Wang, Eldon Geisert**
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We previously observed significant enhanced axonal regeneration in the BXD29-*Tlr4*$^{lps\text{-}2J}$/J (BXD29-*Tlr4*) mouse strain compared with its original cryopreserved BXD29/Ty strain from 1971. This study aimed to identify the specific mutation responsible for the enhanced regeneration observed in the BXD29-*Tlr4* strain. Optic nerve regeneration was induced by knocking down Pten in retinal ganglion cells and triggering a mild inflammatory response using zymosan and CPT-cAMP. The extent of axonal regeneration following optic nerve crush was quantified by counting axons at 0.5 mm and 1 mm from the crush site, and by measuring the travel distance of the five longest axons, as well as the single longest axon. To determine the inheritance pattern of the mutant allele, and map the associated genomic elements, we examined F1 crosses and backcrossed F1 mice to the parental BXD29/Ty strain (F1 Backcross, F1B, n = 34). All F1 mouse (n = 6) displayed significant enhanced axon regeneration compared with the BXD29/Ty strain ($p < 0.01$), demonstrating that the mutation was dominant. However, no significant difference in axon regeneration was observed between mice with homozygous (n = 16) and heterozygous (n = 18) allele at the *Tlr4* locus. Thus, *Tlr4* is not the causative mutation. To identify genetic differences, long-read sequencing was performed on both the BXD29-*Tlr4* and BXD29/Ty strains, revealing 5,155 variants between them. Using these data we have mapped the chromosomes of the F1B crosses to identify the one carrying the mutation. All the chromosomes were excluded as carriers of the mutation with the exception of chromosome 6 and chromosome 16. We are currently sequencing these chromosomes to determine if the changes in axon regeneration is associated with one of these two chromosomes.

## POSTER 07

# INVESTIGATING THE ROLE OF ANGPTL7 GENE MUTATIONS IN MODULATING INTRAOCULAR PRESSURE: A STUDY USING GENETICALLY ENGINEERED NEW ZEALAND WHITE RABBITS

**Kristina Chern, Thomas Tedeschi, Jade Zhang, Charleen Hunt, Thomas Kehrer, Jessica Kuhnert, John Nuara, Craig Grant, Joseph Hickey, Eric Chiao, Brian Zambrowicz, Guochun Gong, Gaurang Patel, Botir Sagdullaev**

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Glaucoma is a multifactorial condition with complex etiology; however, intraocular pressure (IOP) remains the sole modifiable risk factor. Human genetics findings have identified a missense mutation (Gln175His) and a predicted loss of function mutation (Arg177*) in the Angiopoietin-like 7 (ANGPTL7) gene. Patients with these mutations have low IOP and decreased risk for glaucoma. Herein, we sought to validate these human genetics findings by developing a genetically engineered rabbit model.

Angptl7 mutant New Zealand White rabbits were generated using precision single-stranded oligodeoxynucleotide (ssODN)-mediated point mutations targeting exon 3 of the Angptl7 gene through pronuclear injections. Founder rabbits were subsequently bred to produce F1 and F2 generation offspring. Genetic editing was validated using next-generation sequencing and TaqMan assays. IOP measurements were obtained from lightly restrained, unanesthetized rabbits between 9 and 10 am using an iCare Tonovet Plus tonometer. Corneal thickness and anterior chamber depth were assessed using an Aviso Ultrasound Biomicroscopy unit.

Angptl7 heterozygous mutant rabbits demonstrated lower IOP compared to their wild-type littermates. At six months of age, no significant differences were observed in central corneal thickness and anterior chamber depth between wild-type and knockout rabbits.

Altogether, knockout of the Angptl7 gene in the New Zealand White rabbit, informed by human genetic findings, resulted in a reduction of IOP. Despite evident changes in IOP, no significant differences were observed in central corneal thickness and anterior chamber depth in knockout rabbits at six months of age.

## POSTER 08

# DISTINCT TRANSCRIPTOMIC SIGNATURES IN HIGH AND LOW FLOW REGIONS OF THE HUMAN AND MOUSE TRABECULAR MESHWORK

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Outflow resistance in primary open-angle glaucoma is primarily attributed to dysfunction in the juxtacanalicular region of the trabecular meshwork (TM). Outflow through the TM is non-uniform around the eye, i.e., there are distinct high flow (HF) and low flow (LF) regions of the TM. The mechanisms driving this segmental outflow remain unclear, but are likely important for intraocular pressure homeostasis, as glaucomatous eyes exhibit greater variability than healthy ones, and LF regions have lower outflow facility. Here, we investigate transcriptomic differences between HF and LF regions in mouse and human TM using spatial RNA sequencing.

Postmortem human eyes from two donors (1 male, 1 female) were perfused at constant pressure with fluospheres (100 nm) to identify HF and LF regions. Eyes were then perfusion-fixed, and anterior segments were preserved in RNAlater. From each donor, 24 HF and 24 LF sagittal sections (5 μm) were collected, and TM regions were manually outlined for whole transcriptome GeoMx spatial profiling (Nanostring) following established protocols. Additionally, one pair of 8-month-old female C57Bl/6J mouse eyes was perfused in vivo with fluospheres, and 12 HF and LF sections were similarly prepared for spatial profiling. A linear mixed-effect model identified differentially expressed genes, and gene set variation analysis using C2 canonical signaling gene sets (Broad Institute) analyzed enriched pathways between flow regions.

In both mice and humans, HF regions showed increased enrichment of pathways related to extracellular matrix (ECM) components, glycosaminoglycan metabolism, Rho-GTPase activity, TGF-β signaling, and hypoxia compared to LF regions (FDR-adjusted $p < 0.05$). These findings suggest conserved transcriptomic signatures across species and implicate multiple signaling pathways in driving ECM differences between HF and LF regions, potentially contributing to outflow variability. Better understanding of molecular differences between HF and LF regions could inform the development of more targeted IOP-lowering therapeutics.

## POSTER 09

# MODEL OF GLIAL SPECIFIC SIGMA-1 RECEPTOR KNOCKDOWN IN THE MOUSE EYE

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Sigma-1 receptor (S1R) is a small 25 kD transmembrane protein located primarily in the endoplasmic reticulum (ER). S1R plays a role in cellular homeostasis, in part, by modulating calcium signaling, promoting cell survival, and reducing oxidative stress through interactions with various effector proteins. S1R responds to a variety of synthetic ligands and is activated by the drug (+)-pentazocine. Previously, our group has shown that treatment with (+)-pentazocine is neuroprotective in a rat microbead model of glaucoma. Understanding the in vivo cell-specific mechanisms by which S1R affords neuroprotection in glaucoma is complicated by widespread S1R expression in both neurons and glial cells. Until recently, the global S1R knockout mouse was the only transgenic mouse tool available, however, development of the S1R floxed mouse, by our collaborator Dr. Lin Gan, has opened the door for the creation of cell specific S1R knockdown models. In this work, the S1R floxed mouse was crossed with an inducible GFAP-CreERT2 mouse to generate a GFAP-specific S1R knockdown mouse that lacks S1R in astrocytes. Immunohistochemical analysis of S1R in retina combined with analysis of S1R protein expression in retinal lysates and optic nerve head astrocyte (ONHA) cell lysates indicate that S1R is specifically knocked down within GFAP-expressing cells of the mouse visual system. This transgenic mouse model will be a useful tool for studying the astrocyte-specific, S1R-mediated mechanisms by which S1R provides neuroprotection in glaucoma.

## POSTER 10

# OVARIECTOMY MODULATES GENE EXPRESSION OF CELL-ADHESION AND IMMUNE PATHWAYS IN RETINAL GLIAL CELLS AND RETINAL GANGLION CELLS

**Gabriela Sanchez Rodriguez[1], Xinyi Jiang[2], Katie Bales[3], Levi Wood[4], Andrew Feola[5]**

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Menopause is a biological life event that affects aging women that has also been linked to glaucoma (Douglass et al. 2022). Here, we consider the impact of surgically induced menopause (OVX) on gene expression of retinal glial cells and retinal ganglion cells. Brown Norway female rats (3-4 months) were divided to undergo surgical menopause, via ovariectomy (OVX), and were compared to age-matched Naïve animals (n = 6 per group). Retinal tissue was collected 8 weeks after OVX. The glial cells (astrocytes-Muller) and retinal ganglion cells (RGC) were isolated using magnetic-activated cell sorting via anti-GLAST/ACSA-1 and CD 90.1 microbeads from whole retina, respectively (Bales et al., 2022). RNA sequencing was performed to obtain count data. Count data were normalized and differentially expressed genes were identified using DESeq2. Gene set variation analysis was used to identify differentially enriched gene sets. Gene set variation analysis revealed significant upregulation of pathways related to cell adhesion, extracellular matrix organization and immune response in both cell types ($p$-value $< 0.05$). In glial cells, ovariectomy upregulates genes that play a role in cell adhesion (RND3, PCDHB6, ASTN1; $p$-value $< 0.005$). In retinal ganglion cells OVX significantly upregulates genes related to matrix remodeling (MMP23, TIMP1; $p$-value $< 0.001$) and immune function (CD74, SERPING1, C4B, GBP2; $p$-value $< 0.001$). These findings suggest that ovariectomy drives gene expression changes in ECM structure, immune signaling, cell-cell and cell–extracellular matrix interactions. The observed alterations in adhesion-related pathways may influence the ability of cells to interact with their surrounding environment, impacting the cellular response of the retina to injury. Further research is needed to understand how these changes in gene expression are related to developing retinal pathologies.

## POSTER 11

# MITOCHONDRIAL DYSREGULATION IN OPTIC NERVE HEAD ASTROCYTES FOLLOWING BIOMECHANICAL STRAIN AND PIEZO1 ACTIVATION

**Jaclyn Rittershaus[1], Ana Strat[1], Christopher Rudzitis[2], Camille Chin[1], Arunkumar Venkatesan[2], Dan Tso[3], Audrey Bernstein[4], Samuel Herberg[4], Preethi Ganapathy[1]**

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Glaucoma is a progressive blinding disease characterized by optic nerve degeneration. During glaucoma, the optic nerve head (ONH) undergoes biomechanical strain and early metabolic deficits. Local astrocytes are among the first cells to respond to biomechanical strain, and they increase expression of mechanosensitive ion channels such as Piezo1 in models of glaucoma. Physiologically, astrocytes regulate metabolic homeostasis within the ONH. However, not much is known about how biomechanical strain affects the metabolism of ONH astrocytes themselves. Here, we analyzed the effects of tensile strain and Piezo1 activation on primary mouse ONH astrocyte (MONHA) mitochondrial health.

MONHAs were cultured on FlexCell plates and incubated with MitoBrilliant prior to application of 5% static tensile strain for 0-60 m. MOHNAs underwent either strain only, or treatment with Piezo1 agonist, Yoda1 (10 μM), 2h prior to strain. Analysis of mitochondrial morphology revealed that baseline MONHA mitochondria displayed interconnected and tubular morphology. However, with increasing strain duration, mitochondrial connectivity (i.e., total branch length per mitochondrion) and tubularity (i.e., mean form factor) decreased significantly ($p < 0.001$). Interestingly, pre-treatment with Yoda1 blunted these strain-induced changes.

In MONHAs treated for 2h with Yoda1 (10 μM), GsMTx4 (500 nM), co-treatment, or vehicle control, mitochondrial superoxide levels were assessed using MitoSOX, and mitochondrial function was measured via Seahorse. Fluorescence signal intensity analysis revealed that Yoda1-treated MONHAs increased mitochondrial superoxide levels ($p < 0.005$), which was reversed with GsMTx4 co-treatment. Oxygen consumption rates showed that Piezo1 activation increased MONHA basal respiration ($p < 0.05$), ATP synthase-related respiration ($p < 0.0001$), and non-mitochondrial respiration ($p < 0.0001$).

These data suggest tensile strain promotes MONHA mitochondrial fragmentation. Although Piezo1 activation increased mitochondrial reactive oxygen species and activity, Piezo1 activation prior to strain appeared to protect mitochondria from fragmentation. Future studies will assess whether Piezo1 inhibition worsens strain-induced mitochondrial changes, and employ electron microscopy to analyze mitochondrial morphology post strain and Piezo1 activation.

## POSTER 12

# THE EMORY ONH ATLAS: STRUCTURAL BIOMARKERS FOR GLAUCOMA SEVERITY CLASSIFICATION

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We aim to create the first 3D optic nerve head (ONH) atlas that integrates healthy and glaucomatous eyes stratified by disease severity, with the goal of revealing key morphological patterns and progression trajectories in glaucoma. By constructing separate atlases for each severity level and applying supervised UMAP clustering, we aim to improve disease classification and enhance our understanding of optic neuropathies. The atlas was built using optical coherence tomography (OCT) volumes and segmented ONH tissues generated through our custom software, REFLECTIVITY. The dataset included 460 normal eyes, 852 with mild glaucoma (MD > -6 dB), 640 with moderate glaucoma (-6 dB ≥ MD > -12 dB), and 546 with severe glaucoma (MD ≤ -12 dB). Atlas construction was performed using a modified version of IDIR (Implicit Deformation Image Registration), an AI-based iterative framework that combines tissue segmentations and anatomical landmarks to achieve high-precision alignment across subjects. Separate atlases were generated for each disease stage, using the healthy atlas as a baseline reference. Effective strain fields were then computed by deforming glaucomatous eyes into the healthy atlas space, providing localized measures of biomechanical deviation. These strain maps were analyzed using supervised UMAP clustering (80/20 train-test split), which demonstrated clear separation across glaucoma severity groups. The resulting atlases revealed that in mild and moderate glaucoma, minimum rim width was significantly reduced, with strain concentrated along the neuroretinal rim. In contrast, the severe glaucoma atlas showed pronounced cupping and neural tissue loss, with strain localized to the prelaminar region. Regional deformations were most apparent at the nasal neuroretinal rim and anterior lamina cribrosa. Altogether, ONH atlases offer a robust framework for studying structural and biomechanical changes in glaucoma, supporting both large-scale population studies and individualized diagnostics

## POSTER 13

# THE HDAC3/P300-NF-KB REGULATORY AXIS AS A KEY EPIGENETIC MODULATOR OF ABERRANT NUCLEAR DYNAMICS IN GLAUCOMATOUS TM CELL DYSFUNCTION

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Mounting evidence supports that glaucomatous trabecular meshwork (GTM) cells display a persistent pathological phenotype in vitro. We have previously shown that normal TM (NTM) cells respond to glaucoma-associated stressors by increasing nuclear size, suggesting involvement of abnormal nuclear dynamics in TM cell pathobiology. However, the mechanistic underpinnings are largely unclear. Here, we investigate how nuclear architecture and epigenetic landscape impact differentially regulated signaling networks in GTM vs. NTM cells (N = 5) in a physiological soft tissue-like environment using our established ECM hydrogel. Immunostaining and immunoblotting analyses confirmed that GTM cells exhibit increased nuclear volume compared to NTM cells ($p < 0.001$), concurrent with increased H3K9/14 acetylation (euchromatin marker) and decreased H3K27 trimethylation (heterochromatin marker; $p < 0.001$). Notably, this enlarged TM cell nuclear phenotype was also observed in situ in glaucoma patient-derived tissue sections and serial block-face scanning electron microscopy volumes compared to normal tissue, confirming physiological relevance rather than in vitro culture artifact. Epigenetic enzyme assays revealed diverging hyperacetylation mechanisms; total HDAC activity was decreased whereas HAT activity was increased in GTM vs. NTM cells ($p < 0.001$). Specifically, qPCR and immunoblot analyses showed decreased HDAC3 and increased p300 mRNA and protein levels in GTM vs. NTM cells ($p < 0.001$), suggesting that dynamic interplay between HDAC3/p300 modulates H3-hyperacetylation. Pharmacological targeting with the p300 inhibitor garcinol decreased nuclear volume in GTM cells ($p < 0.001$), indicating that hyperacetylation is associated with aberrant nuclear enlargement. Next, we investigated differential signaling pathways associated with these permissive epigenetic changes using paired RNA-seq/ATAC-seq data. Ingenuity Pathway Analyses using 18 differentially expressed genes that also exhibited differentially accessible open chromatin regions showed altered signaling networks involving NFkb-mediated inflammatory processes and CREB/p300-mediated transcription in GTM vs. NTM cells. Together, our data implicate the HDAC3/p300-NFκb regulatory axis as a key epigenetic modulator of aberrant nuclear dynamics in glaucomatous TM cell dysfunction.

## POSTER 14

# THE AUTOPHAGY PROTEIN LC3 INTERACTS WITH COMPONENTS OF THE NUCLEAR CYTOSKELETON IN TRABECULAR MESHWORK CELLS

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Previous work from our laboratory demonstrated the nuclear translocation of the autophagy protein LC3 in response to cyclic mechanical stretch in TM cells. To explore the nuclear functions of LC3 in mechanotransduction, we employed an unbiased proteomics approach to identify its nuclear binding partners. For this, human TM cells were transduced with adenovirus expressing GFP-tagged LC3 (Ad-GFP-LC3) and subjected to cyclic mechanical stretch (20% elongation at 1 Hz) for up to 24 hours. Nuclear fractions were isolated and validated using subcellular markers, followed by co-immunoprecipitation with GFP-Trap® and mass spectrometry analysis. GFP-expressing cells served as controls. We identified 47 nuclear proteins that co-immunoprecipitated with LC3 at levels over threefold higher than with GFP alone ($p < 0.05$). Of these, the top 15 showed more than tenfold enrichment and all contained LC3-interacting region (LIR) motifs. Key interactors included cytoskeletal and trafficking proteins such as EPB4.1, CLTC, CLINT1, E41L2, and SQSTM1. In stretched cells, 33 additional LC3-associated nuclear proteins were detected that were absent in non-stretched or GFP controls; 17 of these also contained LIR motifs. Gene ontology analysis grouped these proteins into two functional categories: nucleolar proteins involved in nuclear processes such as ribosome biogenesis (e.g., nucleophosmin, ribosomal proteins), and nucleoskeletal components associated with mechanotransduction (e.g., MYO1C, LAP2B, EMD). These results reveal novel nuclear roles for LC3, linking it to both structural and regulatory functions in response to mechanical stress. Our findings suggest that LC3 is not only a mediator of autophagy but also a key player in nuclear stress adaptation and mechanobiological signaling in TM cells.

## POSTER 15

# PSEUDO-CENTRAL RETINAL BLOOD VESSEL IN PIG, SHEEP AND GOAT

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Humans and monkeys have central retinal blood vessels (CRBVs) that provide essential vascular supply and drainage to the retina and optic nerve head (ONH). The collagen of pig, sheep and goat ONHs also look like CRBVs. Our goal was to use more comprehensive visualization tools and determine if CRBV-like structures of pig, sheep and goat are true CRBVs, or if they should instead be considered pseudo-CRBVs. We used second harmonic generation image and polarized light microscopy image to visualize the collagen beam structure in the lamina cribrosa (LC) region. We used DAPI staining, multicolor DiOlistics (MuDi) labeling, and GFAP immunostaining to investigate the presence of cell nuclei and astrocytes. We performed DAPI staining and merged with Instant polarized light microscopy (IPOLπ) images to systematically investigate the pseudo-CRBV. With DAPI staining, we found the presence of cell nuclei inside the CRBV-like structures, suggesting that pseudo-CRBV exists. With MuDi labeling, cell-like structures resembling astrocytes are observed in the CRBV-like structures. In contrast, monkeys lack any cells inside this region. With GFAP immunostaining, we found astrocytes within the CRBV-like structures. Notably, more than half of the CRBV-like structures are pseudo-CRBV in sheep and pig. In summary, this study revealed that CRBV-like structure is not merely a vessel but can also contain neural tissue, including astrocytes. Additionally, pseudo-CRBV is not an individual variation but could be a species-wide phenomenon in pigs and sheep. These results highlight the anatomical diversity of the LC across species and suggest the need for further investigation into its functional significance.

## POSTER 16

# STRESS-INDUCED MITOCHONDRIAL FRAGMENTATION IN ENDOTHELIAL CELLS DISRUPTS BLOOD-RETINAL BARRIER INTEGRITY CAUSING NEURODEGENERATION

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The integrity of the inner blood-retinal barrier (iBRB) is crucial for maintaining an optimal microenvironment for neuronal function. However, the contribution of iBRB disruption to glaucomatous neurodegeneration remains poorly understood. Endothelial cells (ECs) rely on a finely tuned balance between fission and fusion to maintain healthy mitochondria. Here, we investigated whether altered mitochondrial dynamics in ECs contribute to the loss of vascular integrity and, if so, what mechanisms underlie mitochondria-related vascular leakage during ocular hypertension (OHT)-induced stress.

OHT was induced by magnetic microbead injection into Endo-MitoEGFP mice, which carry GFP-labeled mitochondria specifically in ECs. We examined vascular integrity using longitudinal fundus fluorescein angiography and Sulfo-NHS-Biotin (550 Da) tracer leakage, followed by stereological retinal sampling. We reconstructed the 3D volume of mitochondria in ECs using Imaris and quantified reactive oxygen species (ROS) levels using MitoSOX. To specifically inhibit mitochondrial fission, we used AAV-mediated expression of a dominant-negative DRP1 selectively in ECs. We increased Claudin-5 (CLDN5) expression in ECs via AAV. We quantified RGC density in RBPMS-stained retinas and assessed retinal-brain connectivity via optomotor reflex responses.

We show that OHT induced mitochondrial fragmentation in retinal capillary ECs accompanied by increased oxidative stress and ultrastructural defects. Analysis of EC mitochondrial components revealed overactivation of dynamin-related protein 1 (DRP1), a central regulator of mitochondrial fission, during glaucomatous damage. Pharmacological DRP1 inhibition or EC-specific in vivo gene delivery of a dominant negative DRP1 mutant was sufficient to rescue mitochondrial volume, reduce vascular leakage, and increase expression of the tight junction claudin-5 (CLDN5). Remarkably, AAV-mediated CLDN5 supplementation improved iBRB integrity, promoted RGC survival, and rescued visual behaviours.

Our findings reveal that preserving mitochondrial homeostasis and EC function are valuable strategies to enhance neuroprotection and improve vision in glaucoma.

## POSTER 17

# INVESTIGATING THE ROLE OF NETRIN1/DCC SIGNALING IN MAINTAINING RGC INTEGRITY DURING GLAUCOMA PATHOLOGY

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Glaucoma pathology involves complex gene dysregulation in the retinal ganglion cells (RGCs). Identifying the critical molecular changes in degenerating RGCs will help us better understand the pathogenesis of the disease. Genetic studies in mice have shown that the signaling pathway elicited by the Netrin1 ligand and its DCC receptor is required for the survival of developing mouse RGCs. Netrin1/DCC activation also promotes survival and axon growth in induced human RGCs. In addition, deletion of the chromosome region encompassing DCC (18q21.2 locus) in humans is found to be associated with retina and optic nerve head abnormalities. However, whether Netrin1/DCC plays a role in maintaining RGC integrity during adult stage is completely unknown. To test this, we conditionally knocked out DCC gene using intravitreal injection of AAV-Cre in adult mouse retina, and found that adult onset loss of DCC impairs RGC survival and synaptic connections. Using retinal samples from human individuals, we also found that DCC reduction is closely associated with glaucoma pathology. To further investigate the role of DCC activity in maintaining RGC integrity in adults, we also overexpressed Netrin1/DCC in adult mouse retina using AAV vectors, before inducing RGC death with the microbead occlusion model. We found that activating the signaling reduces RGC death in the mouse model of glaucoma. Together, our results suggest that Netrin1/DCC signaling is likely to have a key role in maintaining RGC integrity in adult stage, and our future studies will further investigate this hypothesis.

## POSTER 18

# THE ROLE OF MULLER GLIA CALCIUM AND PURINERGIC SIGNALING IN THE RESPONSE TO OCULAR HYPERTENSION

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Ocular hypertension (OHT) stress induces glaucomatous degeneration in the retina mediated through neuroinflammation, a response which is dependent on the transient receptor potential vanilloid (TRPV4) mechanosensitive channel and the release of cytokines. However, the specific mechanisms underlying this, the links between TRPV4 and inflammatory responses, and the role of specific retinal cell types in this process are not fully understood.

Muller glia are unique retinal glia which are critical to the regulatory environment supporting RGCs. Under normal IOP fluctuations, they provide structural support and help to promote neural survival. However, when these responses pass pathological thresholds, they can trigger gliosis reactivity and proinflammatory signaling, putting greater metabolic and environmental strain on retinal ganglion cells. In keeping with these mechanosensitive roles, Muller glia express mechanically sensitive ion channels, including TRPV4, which are permeable to cations, primarily $Ca^{2+}$. Excess $Ca^{2+}$ influx through TRPV4 has been shown to induce harmful responses in microglia and astrocytes through extracellular ATP and phospholipase signaling.

We evaluated whether the response of Muller glia to mechano-stress is dependent on TRPV4-driven increases in intracellular Ca2+ and extracellular ATP, linked to increased pro-inflammatory signaling through cytokine release.

This was investigated by real-time imaging of calcium dynamics using Fura-5F AM, ATP signaling using HEK293 “sniffer” cells overexpressing P2X2-GCaMP fluorescent ATP receptors, and cytokine release response of isolated Muller glia and RGCs using a broad inflammatory cytokine antibody array.

We found that Muller glia exhibited consistent calcium influx response to TRPV4 activation whereas RGCs had both responsive and unresponsive subtypes. Further, isolated RGCs and Muller glia exhibited particular cytokine responses to TRPV4 stimulation and mechanical stress. Characterization of the ATP signaling dynamics, and how this links the calcium signaling to cytokine release in Muller glia is currently underway.

## POSTER 19

# GLAUCOMA-RELATED REDUCTION IN TUBERAL-INFERIOR HYPOTHALAMIC VOLUME ALTERS HORMONAL REGULATION OF METABOLIC FUNCTION

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Emerging evidence from animal models suggests that retinohypothalamic neurodegeneration in experimental glaucoma may contribute to systemic metabolic dysregulation. However, its clinical relevance to glaucoma patients remains unclear. In a structural equation model of 822 participants, we tested whether reduced hypothalamic volume in glaucoma is associated with blood-based metabolic markers, including hormonal regulators. We found that, compared to age-matched healthy subjects, glaucoma patients had significantly lower total hypothalamic volume, which in turn predicted reduced levels of insulin-like growth factor 1 (IGF-1) and testosterone. Among these, reduced testosterone robustly mediated adverse metabolic outcomes, including increased lipid burden (LDL, apolipoprotein B), reduced cardioprotective lipids (HDL, apolipoprotein A), and poorer glycemic control (glucose, HbA1c). Control analyses using lateral geniculate nucleus (LGN) volume, a visual relay structure unrelated to metabolic regulation, confirmed the specificity of these associations: while LGN volume was reduced in glaucoma, it showed no direct or indirect association with metabolic outcomes. When hypothalamic subregions were examined, only the tuberal-inferior hypothalamus exhibited a significant mediation pathway, showing a significant reduction in glaucoma, prediction of lower blood IGF-1 and testosterone levels, and associations with increased lipid burden, reduced cardioprotective lipids, and impaired glycemic control. Other hypothalamic subregions - including anterior inferior, anterior superior, tuberal superior, and posterior volumes - showed weak or inconsistent associations with hormones and no reliable effects on metabolic markers. These results pinpoint the tuberal-inferior hypothalamus as a key node linking glaucomatous neurodegeneration to hormone-related metabolic vulnerability. This study identifies a novel brain–body pathway in glaucoma, suggesting that localized hypothalamic atrophy may impair endocrine regulation of both lipid and glucose metabolism.

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## POSTER 20

# IP3R1-DEPENDENT EXCITATION INSTABILITY CONTRIBUTES TO RETINAL GANGLION CELL DYSFUNCTION IN EXPERIMENTAL GLAUCOMA

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Excitation instability in retinal ganglion cells (RGCs) has been observed following optic nerve injury; however, the molecular mechanisms underlying this phenomenon and its pathological relevance in glaucoma remain poorly defined. Inositol 1,4,5-trisphosphate receptor type 1 (IP3R1)-mediated $Ca^{2+}$ release from the endoplasmic reticulum and mitochondria is a key regulator of neuronal excitability and energy homeostasis. Here, we investigated the role of IP3R1 in mediating RGC excitation instability, metabolic integrity and survival under ocular hypertension (OHT)-induced stress.

OHT was induced in adult mice by intracameral injection of magnetic microbeads. RGC-specific stimulation of IP3-mediated $Ca^{2+}$ release was achieved via adeno-associated virus encoding the Gq-coupled DREADD hM3Dq (AAV.hM3Dq), activated by systemic clozapine-N-oxide (CNO, 5 mg/kg i.p.). IP3R1 expression was selectively silenced using siRNA (siIP3R1). Two-photon laser scanning microscopy (TPLSM) was used to longitudinally monitor single-cell $Ca^{2+}$ dynamics in living Thy1-GCaMP6f mice. Entropy, a quantitative metric of signal variability, was computed to assess excitation instability. Intracellular ATP levels were assessed using the FRET-based biosensor ATeam delivered via AAV in vivo.

OHT significantly increased spontaneous $Ca^{2+}$ transient variability in RGCs (sham: 4.85 ± 0.04 log2; OHT: 5.77 ± 0.19 log2; N = 4-8 mice/group, n = 98-102 RGCs/group; $p < 0.01$), indicating higher excitation instability (entropy). Following CNO-mediated activation of hM3Dq, glaucomatous RGCs displayed exacerbated $Ca^{2+}$ dysregulation ($n > 700$ RGCs; $p < 0.01$). This instability was accompanied by marked ATP depletion (sham: 0.95 ± 0.03; OHT: 0.72 ± 0.03; $p < 0.01$), reflecting compromised metabolic integrity. Multi-modal molecular analyses including scRNA-seq, qRT-PCR, flow cytometry, and immunohistochemistry confirmed significant downregulation of IP3R1 in glaucomatous RGCs. Importantly, siIP3R1 further exacerbated both $Ca^{2+}$ transient variability and RGC death under OHT conditions.

These findings reveal that IP3R1 dysfunction promotes excitation instability, energy deficits, and RGC degeneration in glaucoma. Targeting this pathway may offer novel opportunities for neuroprotection in early-stage disease.

## POSTER 21

# STABILIZED 21OLF SCAFFOLD ENABLES BIOCHEMICAL, BIOPHYSICAL, AND STRUCTURAL CHARACTERIZATION OF SEVERE OLF-MYOCILIN MUTANTS

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Missense mutations in the MYOC gene, mostly within its olfactomedin (OLF) domain, are responsible for a subset of primary open-angle glaucoma (POAG) cases. Limited information is available for the most severe mutants because the resulting mutant proteins are too destabilized for biochemical and structural characterization. To address this challenge, we took inspiration from the field of protein design: we introduced severely destabilizing mutations (P370L, Y437H, I499F, and W286R) into an engineered OLF scaffold (21OLF) characterized previously using the PROSS algorithm. 21OLF(WT) retains the wild-type fold but is 20°C more stable than the original wild-type OLF scaffold. 21OLF(WT) can better tolerate severely perturbing mutations for biochemical and structural of the corresponding mutants. While mutants retained partial calcium binding, 21OLF(P370L) did not. Crystal structures of 21OLF(P370L), 21OLF(Y437H), and 21OLF(I499F) reveal distinct local and global structural changes, and changes in dynamics are observed by biomolecular NMR. Despite enhanced stability, 21OLF mutants retained aggregation propensity when incubated at Tm -7°C, showing faster and more complex aggregation kinetics than WT OLF. Combined, these findings provided insights into how mutations impact the OLF structure, representing an important first step toward understanding how these mutations perturb OLF to elicit a severe disease phenotype.

## POSTER 22

# DEGENERATION OF TARGET-SPECIFIC IPRGC CIRCUITS UNDERLIES NON-IMAGE-FORMING DEFICITS IN GLAUCOMATOUS MICE

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We investigated how glaucoma affects light-induced sleep, cognition, and mood functions through intrinsically photosensitive retinal ganglion cells (ipRGCs) pathways. Laser photocoagulation of the corneal limbus was used to induce sustained elevation of intraocular pressure (IOP) in mice. First, the inducible Opn4Cre-ERT2/Cre-ERT2 mice were crossed with R26Syp-tdT/+ mice to accurately map ipRGC projections in the adult brain. We analyzed the glaucoma-associated alterations in ipRGC innervation of the suprachiasmatic nucleus (SCN) and perihabenula (PHb), regions involved in circadian pacemaking and light-induced mood regulation, respectively. In addition, we injected viral tracers into brain targets of interest to characterize the survival of target-specific ipRGCs in the retina in glaucoma. Next, mice with bilaterally elevated IOP ( ≥ 20 mmHg) for more than 3 months were assessed behaviorally. Light-induced anxiety-like behavior was measured using elevated plus maze (EPM), while circadian photoentrainment and negative masking behavior were evaluated through actogram recordings of running wheel activity under different light-dark conditions. We observed disruptions in ipRGC circuitry in glaucomatous mice, accompanied by altered light-induced anxiety-like behaviors and abnormal masking responses. These findings reveal changes in the non-image-forming visual pathways affected by glaucomatous damage. Our subsequent investigations will explore whether manipulating ipRGCs would recapitulate or rescue non-image-forming deficits in glaucoma and unveil the underlying mechanisms involved.

## POSTER 23

# TENSIN 3: A NOVEL TARGET FOR CONTROLLING TISSUE STIFFNESS AND CONTRACTILITY

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Tensin3 (TNS3) is a focal adhesion mechanosensing protein that links the extracellular matrix (ECM) to the actin cytoskeleton mediating outside-in and inside-out signaling. It plays a central role in promoting fibrillar adhesion formation alongside Talin-1 and integrin α5β1, which in turn drives the deposition of insoluble fibronectin in the ECM and supports the formation of stress fibers inside the cell. This study identifies TNS3 as an important regulator of tissue stiffness and cellular contractility through its modulation of fibrillar adhesions. In western blot analysis, TNS3 expression was significantly elevated in human trabecular meshwork (HTM) cells following treatment with intraocular pressure (IOP) disruptors including endothelin-1 ($p = 0.03$), TGFβ2 ($p = 0.03$), and dexamethasone ($p = 0.004$), suggesting a role in stress-induced TN3 remodeling. To elucidate TNS3 function, siRNA-mediated TNS3 (siTNS3) knockdown and constitutive expression via adenovirus (AdTNS3) in HTM were performed. siTNS3 reduced actin stress fiber formation, while AdTNS3 increased actin polymerization, indicating the regulatory role of TNS3 in actin dynamics. TNS3 overexpression also enhanced integrinβ1 activation ($p = 0.04$) and Talin-1 expression ($p = 0.01$), both confirmed by western blot and immunofluorescence, which showed increased membrane localization. Immunoprecipitation demonstrated an interaction between TNS3 and Talin-1, suggesting a coordinated role in adhesion maturation. Correspondingly, fibronectin fractionation assays revealed greater levels of insoluble fibronectin upon TNS3 induction, reflecting enhanced ECM assembly and potential stiffening. Importantly, phosphorylated myosin light chain levels were elevated in AdTNS3 treated cells, indicating increased contractile activity. Together, these findings establish TNS3 as a critical regulator of cytoskeletal tension and ECM remodeling in the TM. By promoting fibrillar adhesion assembly and cellular contractility, TNS3 contributes to tissue stiffening, a hallmark of fibrotic and glaucomatous pathology. Targeting TNS3 and its interactors may therefore offer novel therapeutic strategy for treating elevated IOP and the progression of glaucoma.

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## POSTER 24

# ROLE OF IL-6/STAT3 PATHWAY IN GLAUCOMATOUS IRISES AND ITS ASSOCIATION WITH FILTERING SURGERY OUTCOMES

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Glaucoma is associated with immune-mediated mechanisms contributing to retinal ganglion cell loss. Despite growing recognition of the immune system's involvement, little is known about the immunological milieu within ocular tissues, particularly the uvea. This study investigates the expression patterns of inflammatory mediators, with a focus on the IL-6/STAT3 axis, in the irises of glaucoma patients to uncover mechanistic insights into disease progression and surgical outcomes.

Iris tissues were obtained from patients with primary open-angle glaucoma (POAG), primary angle-closure glaucoma (PACG), and secondary glaucoma - including uveitic, neovascular, and trauma-related cases - and compared with cadaveric controls. The expression of IL-6, its receptor components (IL-6R and gp130), STAT3, and other genes involved in proliferation, apoptosis, and fibrosis was analyzed by Real-Time PCR on the RNA extracted from these tissues.

IL-6/STAT3 signaling components were consistently downregulated in both primary and secondary glaucoma groups. Altered expression of fibrotic marker (α-SMA) and apoptosis regulators (BAX and BCL2) suggested a unique immune signature within the glaucomatous iris microenvironment. Additionally, a three-month clinical follow-up showed a trabeculectomy success rate of over 94%, suggesting a possible link between positive surgical results and cytokine-mediated immunological homeostasis.

These results imply that cytokine homeostasis may affect the effectiveness of surgical procedures intended to control intraocular pressure and provide new insights into the function of the IL-6/STAT3 axis in regulating immune responses within the iris during glaucoma.

## POSTER 25

# SUBSTRATE MECHANICS DIFFERENTIALLY AFFECT THE STIFFNESS OF PRIMARY HEALTHY AND GLAUCOMATOUS HUMAN TRABECULAR MESHWORK CELLS

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The trabecular meshwork (TM) regulates aqueous humor outflow by remodeling its extracellular matrix. In glaucoma, the juxtacanalicular (JCT) aspect of the TM, which is a primary site of outflow resistance, becomes significantly stiffer. Here, we used atomic force microscopy (AFM) to examine how primary TM cells respond to varying substrate stiffness.

We fabricated collagen I–coated silicone elastomer substrates with Young's moduli of 3, 15, 30, and 65 kPa to mimic the progressive stiffening of the glaucomatous microenvironment. We cultured healthy and glaucomatous TM cells as single-cell populations. Using AFM with 5 µm spherical tips, we measured cytoplasmic stiffness and analyzed differences using ANOVA followed by the Steel-Dwass post hoc test.

In healthy TM cells, the average Young's modulus increased with substrate stiffness: 1.18 ± 0.075 kPa (3 kPa), 1.46 ± 0.2 kPa (15 kPa), 1.5 ± 0.12 kPa (30 kPa), and 1.85 ± 0.16 kPa (65 kPa), with a significant difference between 3 kPa and 65 kPa conditions ($p < 0.001$). In contrast, glaucomatous TM cells exhibited consistently lower stiffness: 0.51 ± 0.04 kPa (3 kPa), 0.86 ± 0.05 kPa (15 kPa), 0.87 ± 0.07 kPa (30 kPa), and 0.86 ± 0.06 kPa (65 kPa). A significant difference was observed only between the 3 kPa and stiffer substrates ($p < 0.001$), with no further increases beyond 15 kPa. At all substrate stiffnesses, glaucomatous cells were significantly softer than healthy cells cultured under the same conditions ($p < 0.001$).

These results suggest that healthy TM cells are more mechanically adaptive to increases in substrate stiffness, while glaucomatous TM cells exhibit impaired mechanoadaptation. The reduced stiffness of glaucomatous cells across all conditions may reflect altered cytoskeletal dynamics and mechanosensitivity that can contribute to glaucoma pathophysiology.

## POSTER 26

# ASTROCYTE AND MÜLLER GLIA REACTIVITY IN RESPONSE TO ACUTE INTRAOCULAR PRESSURE ELEVATION IN THE LIVING HUMAN MACULA

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In animal models of acutely elevated intraocular pressure (IOP), macroglia, including astrocytes and Müller glia, become reactive. TGF-β is a regulator of macroglial reactivity, and connexin-43 (Cx43) mediates communication between macroglia facilitating gliosis, and altering GFAP and vimentin levels in the retina. This study evaluates macroglial reactivity spatially across the macula following acute IOP elevation in the living human eye.

Three research-consented brain-dead organ donors underwent screening for inclusion criteria and one eye was randomly selected to undergo IOP elevation while the contralateral eye was maintained at physiological levels. The treated eye was exposed to prolonged IOP elevation achieved by injection of viscoelastic gel into the anterior chamber. This injection resulted in a sustained IOP elevation (mean IOP across follow-up: 41.46 mmHg) for 8 to 9 hours until organ procurement. Eyes were subsequently dissected, formalin-fixed and maculas were parrafin embedded. Maculas were sectioned at 5 µm and underwent immunohistochemistry, RNA in-situ hybridization, and spatial transcriptomics. Mean RNA expression and surface area of macroglial intermediate filaments were compared between treated and control eyes in 6 parafoveal regions and in the fovea.

In our initial two donors, there was greater RNA expression (represented as puncta per nuclei) for TGFB1 ($\Delta$ = 1.16 ± 0.58) in the outer nuclear layer within the inferior nasal macula, and an increased vimentin surface area ($\Delta$ = 182.57 ± 150.19 µm$^2$) in the superior fovea in treated eyes. In the inner nuclear layer, we found an increase in RNA expression for GJA1 (Cx43) ($\Delta$ = 1.79 ± 0.38) in the superior fovea and a decrease in GFAP surface area ($\Delta$ = -206.99 ± 93.65 µm$^2$) in the superior temporal macula. Preliminary spatial transcriptomics from one donor implicate transcripts involved in glial neurotransmitter uptake and metabolism and TGF-β signaling. These results support macroglial reactivity to acute IOP elevation with regional and layer specificity in the human macula with implications for chronic IOP elevation and disease.

## POSTER 27

# ROLE FOR CAVEOLIN-1 IN IOP HOMEOSTASIS AND OUTFLOW VESSEL TONE INDEPENDENT OF ROCK INHIBITION OR ISOFLURANE IOP LOWERING EFFECTS

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Genome-wide association studies have linked common variants near the CAV1/CAV2 gene loci to risk for elevated intraocular pressure (IOP) and primary open-angle glaucoma. The importance of caveolin-1 (CAV1) in IOP homeostasis was observed in global and endothelial CAV1 knockout (KO) mice that exhibit elevated IOP, increased eNOS activity, and enlarged distal venules (DV) in the conventional outflow pathway. Potential mechanisms underlying these effects were assessed following mass spectrometric evaluation of the CAV1 interactome following mechanical stretch (mimicking elevated IOP) of cells from the proximal part of the conventional outflow pathway (including trabecular meshwork and Schlemm's canal cells). Consistently, Rho-associated coiled-coil kinase-1 (ROCK1) was found associated with CAV1 after mechanical stretch. To examine whether CAV1 modulates IOP through the RhoA/ROCK signaling pathway, we assessed the effects of ROCK inhibition (RKI) on IOP and DV tone in global CAV1 KO mice. CAV1 KO mice show enlarged DVs at baseline, with further dilation and a reduction in IOP following RKI treatment. Additionally, extended isoflurane anesthesia lowers IOP, however IOP effects were reduced in CAV1 KO mice. Thus, it appears that RKI and isoflurane act downstream or through concurrent mechanisms modulating IOP independent from the CAV1 scaffold, likely through modulation of eNOS activity and cytoskeleton dynamics. The reduced IOP response to isoflurane in CAV1 KO mice supports CAV1 involvement in outflow responsiveness, potentially through nitric oxide desensitization and tolerance.

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## POSTER 28

# IN TOTO VASCULAR LABELING OF THE NON-HUMAN PRIMATE AND HUMAN OPTIC NERVE HEAD

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The optic nerve head (ONH) contains a dense network of vasculature which provides critical support to metabolically-demanding neural tissues within it. A better understanding of the vascular architecture within this vulnerable region will open doors to mechanistic insights, detection, and treatment of glaucoma. Although many techniques have been deployed towards imaging ONH vasculature, it has remained a challenge to visualize network structures with capillary-level detail in an intact condition. Our goal was to uncover the 3D microstructure of intact vasculature within the non-human primate and human ONH. ONH tissue of rhesus macaque (3), marmoset (3), and human donor (1) eyes were fixed, permeabilized, and vasculature was immersion-labeled with fluorescently-tagged lectins. Tissues were incubated in an ascending alcohol gradient, optically cleared with benzyl alcohol/benzyl benzoate, and held in ethyl cinnamate. Volumetric images were collected via multiphoton and confocal microscopy before and after clearing. Our tissue preparation approach provided a robust method to substantially increase optical transparency in non-human primate and human donor ONH samples. Vasculature was discernible and could be imaged at high resolution throughout its thickness, tested as deep as 850μm. In toto immersion-labeling facilitated visualization of vascular network structure, including within deep regions of the ONH such as the collagenous lamina cribrosa and potential ONH interpericyte tunneling nanotubes (IP-TNTs). Immersion-based rather than perfusion-based vascular labeling can reveal critical elements of vascular structure external to the vascular lumen, including pericytes and potential IP-TNTs. IP-TNTs are key mediators of vascular regulation in the retina, with established structural and functional changes in a mouse model of glaucoma. The presence of IP-TNTs in the primate ONH has important implications for the vascular dynamics of this region. Future work is needed to understand the network-level effects of ONH vasculature and associated elements that influence local blood flow in health and glaucoma.

## POSTER 29

# MKK4 AND MKK7 ARE REQUIRED FOR RETINAL GANGLION CELL AXONAL MAINTENANCE LONG-TERM

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During glaucomatous neurodegeneration, an initial insult to retinal ganglion cell (RGC) axons leads to activation of molecular pathways that result in RGC death. Our lab and others have shown that the molecular pathways underlying the death of RGC somas and axons are distinct, and thus RGC death is compartmentalized. Further investigations have searched for a common molecular regulator of axonal and somal RGC death; one that could link the initial axonal injury to the compartmentalized RGC death pathways. Recent work suggests that MAP2Ks MKK4 and MKK7 (MKK4/7) hold potential. After optic nerve crush in mice (an acute, glaucoma-like axonal injury), dual deficiency of Mkk4/7 led to prolonged RGC somal protection, axonal protection, and functional protection. However, MKK4 and MKK7 have also been linked to homeostatic functions in retinal development and throughout the nervous system in adults, bringing into question whether MKK4/7 have roles in adult retinal neuronal maintenance. To investigate this, we used intravitreally delivered AAV2-CMV-CRE-GFP to remove Mkk4/7 in adult RGCs and aged the mice to 8 months following injection. Dual deficiency of Mkk4/7 in mouse RGCs for 8 months led to no changes in RGC somal counts, nor in pattern electroretinography amplitudes, suggesting that RGC somas can survive long-term without these molecules ($P > 0.3$ for all tests). However, as early as one month following the loss of Mkk4/7, unusual TUJ1+ formations began to appear in the ganglion cell layer. Additionally, 8 months of Mkk4/7 dual deficiency led to a stark reduction in visually evoked potential amplitude ($p < 0.005$; $n \geq 10$) and axonal degeneration (as judged by PPD staining) compared to wildtype retinas. Together, these data suggest that MKK4/7 have critical roles in RGC neuronal maintenance, particularly in the RGC axon. Furthermore, these data bring into question whether treatment strategies targeting MKK4/7 function in RGCs would be viable long-term.

## POSTER 30

# TREM2 DEFICIENCY ALTERS MICROGLIA RESPONSE TO OPTIC NERVE CRUSH

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Glaucoma is a neurodegenerative disease characterized by retinal ganglion cell (RGC) death and dysfunction. Microglia, resident immune cells of the CNS, survey healthy tissue for signs of damage or distress then respond by releasing critical signaling molecules, clearing debris, and regulating synapses. In diseased tissue, these activated microglia can be neurotoxic or neuroprotective. Studies that have altered the number or activity of microglia demonstrate that activated microglia modify glaucomatous degeneration in mouse models, but the precise molecular mechanisms involved are poorly understood. Disease Associated Microglia (DAM) are an activated microglia phenotype implicated in many neurodegenerative diseases. Canonically, the phenotype is driven by activation of Triggering Receptor Expressed on Myeloid cells 2 (TREM2). In the context of Alzheimer's Disease, DAM protect neurons by compacting amyloid plaques and clearing debris. TREM2 is also upregulated in the diseased optic nerves of DBA/2J mice, suggesting a role in glaucoma, though it remains unclear what that role is. We performed optic nerve crush on wildtype and Trem2 deficient mice to determine whether activated microglia in the retina respond to a glaucoma-relevant injury in a TREM2-dependent manner. Three days after optic nerve crush, Trem2 deficient microglia in the inner plexiform layer are less activated and are less phagocytic as judged by IBA1 surveillance analysis ($n = 3$ per group; $p < 0.0025$) and CD68 immunofluorescence ($n = 3$ per group; $p < 0.024$) compared to the wildtype controls. Further, transcriptional data showed a relative increase of interferon signaling (IFITM3; $n = 3$-5 per group, $p < 0.028$) in the Trem2 deficient retinas. These changes suggest that the activated microglia population is sensitive to activation of the TREM2 pathway after an axonal injury, particularly surrounding RGC synapses and dendrites.

## POSTER 31

# MODELING OPTIC NERVE GLIOMA FORMATION AND AXONAL DEGENERATION USING A HUMAN IPSC-DERIVED COMPARTMENTALIZED MICROFLUIDIC PLATFORM

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Optic nerve degeneration is a hallmark of various retinal pathologies, yet the underlying mechanisms remain poorly understood. To interrogate the role of neuron-glia interactions in axonal vulnerability, we developed a human stem cell-based, compartmentalized microfluidic platform that isolates retinal ganglion cell (RGC) somas from their axons, enabling precise spatial control over cellular environments. In this three-chamber system, human induced pluripotent stem cell (iPSC)-derived RGCs extend axons from a soma-containing chamber through microgrooves into adjacent chambers, where they can be selectively co-cultured with astrocytes or exposed to engineered extracellular conditions. This architecture facilitates targeted studies of glial modulation of axonal integrity. When RGCs were cultured with patient-derived astrocytes carrying a pathogenic mutation associated with neurofibromatosis type 1 (NF1), we observed significant reductions in neurite complexity and electrophysiological activity as measured by multi-electrode array (MEA). The introduction of these patient-derived astrocytes into the axonal compartment induced distinct axonal morphological changes and signs of axonal stress, suggesting deleterious glial signaling in a disease-relevant context. Bulk RNA sequencing revealed transcriptional signatures consistent with disrupted neuronal and glial homeostasis and axonal degeneration. To further explore the impact of biomechanical stress, current efforts are refining these approaches to engineer a central chamber hydrogel system embedding iPSC-derived RGC axons and astrocytes within tunable 3D matrices. This allows modulation of tissue stiffness, which has been linked to astrocyte reactivity and axonal pathology. Together, this modular platform provides a physiologically relevant system to dissect disease mechanisms underlying optic nerve degeneration and to enable future therapeutic testing.

## POSTER 32

# ESTABLISHING AN IN VITRO MODEL OF EYE-TO-BRAIN CONNECTIVITY USING HUMAN PLURIPOTENT STEM CELLS

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Glaucoma and other optic neuropathies affect millions of people each year, leading to progressive visual loss and blindness. Optic neuropathies are termed as such because the primary injury to cells occurs to the axons of the optic nerve. This induces progressive axon dieback and eventual death of retinal ganglion cells (RGCs). There is currently no known method to completely regenerate these long axons, making restoration of the eye-to-brain connection one of the largest barriers to regeneration of the visual system. Importantly, there is also no established in vitro model of RGC to brain connectivity. Thus, we aim to create a functional eye-to-brain model on a chip using human pluripotent stem cells (hPSCs). hPSCs were directed to differentiate into RGCs, thalamic neurons, or glia, and the orientation of these cells along the optic pathway was established in a microfluidic-based system. This 3-chamber system allows the study of each cell type and its interactions with RGCs in a compartmentalized nature. For example, oligodendrocytes were grown along RGC axons, while thalamic cells were grown at RGC terminals to encourage synaptic contacts. We show that when RGCs were grown in the presence of thalamic neurons, RGC axons exhibited more rapid and directional growth. Further, the presence of oligodendrocytes along RGC axons enhanced oligodendrocyte maturation as evidenced by increased branching and expression of myelin basic protein in oligodendrocytes. Ongoing experiments aim to determine whether oligodendrocytes also alter RGC complexity. These results represent the first application of hPSC-derived RGCs in a manner that effectively recapitulates their highly compartmentalized properties, as well as the use of microfluidic platforms to model eye-to-brain connectivity in a dish. Taken together, these results should profoundly impact future studies, providing a much more physiologically relevant in vitro model for the development and degeneration of the optic pathway.

## POSTER 33

# ROLE OF INTEGRIN ALPHA 4 AND ALPHA 9 IN FN+EDA-INDUCED TRABECULAR MESHWORK DAMAGE AND OCULAR HYPERTENSION

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Fibronectin containing the extra domain A (FN+EDA) is a known activator of toll like receptor 4 (TLR4) signaling. We have identified FN+EDA activated TLR4 initiates a fibro-inflammatory response in the trabecular meshwork (TM) causing elevated intraocular pressure (IOP) and TM damage. The molecular basis of how FN+EDA activates TLR4 is not completely understood. Here, we tested the hypothesis that α4β1 and/or α9β1 integrins act as co-receptors in prolonged FN+EDA induced activation of TLR4 signaling, leading to TM damage. Primary human TM cells were pre-treated with blocking antibodies to integrin α4, α9, or with mAb IgG as a negative control for 30 mins and then either plated on wells pre-coated with cellular fibronectin (cFN) which contains EDA isoform or treated with TGFβ2 (N = 3 independent cell strains). Western blot analysis after 24 hours showed total FN, FN+EDA, and integrin α4 expression was significantly increased with TGFβ2 treatment ($p < 0.05$). Integrin α9 expression was significantly increased with both cFN and TGFβ2 treatments ($p < 0.05$). Blocking integrin α4 significantly reduced cFN induced FN+EDA expression (two-way ANOVA, $p < 0.05$). Blocking integrin α4 or α9 significantly reduced TGFβ2 induced FN+EDA and total FN expression compared to controls (two-way ANOVA, $p < 0.05$). Conditional knockdown of integrin α4 and α9 was performed by intravitreal injection of Ad5.Cre in one eye of EDA+/+α4fl/fl (N = 16) or EDA+/+ α9fl/fl (N = 11) mice, which constitutively express FN+EDA and contain α4 or α9 specific LoxP sites respectively. Eyes were injected at 12-13 weeks of age as we previously demonstrated that EDA+/+ mice develop elevated IOP by 14 weeks of age. The control eyes were injected with Ad5.Null or kept un-injected. Knockdown of either integrin α4 or α9 blocked the EDA-induced ocular hypertension compared to control eyes (one-way ANOVA, $p < 0.05$). The results indicate integrin α4β1 and α9β1 are important regulators of FN+EDA induced activation of TLR4 and TM damage.

## POSTER 34

# IOP-LOWERING EFFICACY OF A TOPICAL EP2 RECEPTOR AGONIST IN FELINE GLAUCOMA

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Glaucoma is a leading cause of blindness and enucleation in domestic cats, and is typically associated with elevated intraocular pressure (IOP). However, safe and effective medical treatment options for cats with glaucoma are currently limited. This study aims to evaluate the IOP-lowering efficacy of topical prostaglandin E2 receptor 2 (EP2) agonist, omidenepag isopropyl (OMDI), and effects on pupil diameter (PD) in cats with glaucoma.

In this randomized, placebo-controlled, masked pilot study, eight LTBP2 mutant cats with congenital glaucoma received a drop of 0.002% OMDI (Eybelis®, Santen Pharmaceutical Co., Ltd., Japan) in one eye and saline in the contralateral eye. IOP (TonoVet, iCare) and PD (digital caliper) were measured by a masked observer prior to, and every 2 hours until 12hrs- then at 24hrs-post-OMDI administration. Paired t-test and repeated measures ANOVA were used for statistical analyses and $p < 0.05$ considered significant.

There were no significant between-eye differences in IOP or PD prior to the treatment phase ($p = 0.64$ and 0.74, respectively). OMDI-treated eyes exhibited a significant reduction in IOP compared to saline-treated eyes at 2-, 4-, and 6-hours post-administration, with mean IOP reduction of 6.8 mmHg (~40% reduction relative to contralateral control) across this time period. Maximum IOP reduction was observed at 2 hours post-OMDI administration (mean IOP = 9.1 ± 2.9 mmHg) compared to the contralateral saline-treated eyes (mean IOP = 23.4 ± 8.7 mmHg, $p = 0.0011$). No differences in PD were observed between OMDI- and saline-treated eyes.

Topical 0.002% OMDI significantly lowers IOP without inducing miosis in cats with glaucoma compared to saline-control. Our findings provide promising evidence supporting topical selective EP2 agonists as a treatment for feline glaucoma and will inform dosing frequency for a planned follow-on, longer-term treatment study in our spontaneous feline glaucoma model.

Support: Vision for Animals Foundation, Research to Prevent Blindness, NIH P30EY0016665

## POSTER 35

# A COMPREHENSIVE SET OF PATHWAY-WIDE POLYGENIC RISK SCORES TO FACILITATE PRECISION GLAUCOMA CARE

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Primary open-angle glaucoma (POAG) is a complex-inherited disease with hundreds of risk loci identified to date. Intriguingly, genes in many of these loci function in disease-relevant biological pathways. We hypothesize that differences in the specific biological drivers of disease in any given individual may in part contribute to heterogeneity in what is clinically treated as POAG. To begin testing this hypothesis, we have generated a comprehensive set of pathway-specific POAG polygenic risk scores (PRSs) and tested their predictive power among European (EUR) and African (AFR) individuals in the All of Us (AoU) Research Program. POAG case/control cohorts were generated using ICD-9/10 diagnosis codes and subdivided by genetically predicted ancestry (EUR: 1846 cases/84,654 controls; AFR: 1042 cases/15,966 controls). Genome-wide PRS weights were generated using Lassosum penalized regression trained in AoU. Pathway-specific PRSs were then generated for all 50 MSigDB Hallmark Gene Sets using Lassosum weights of all variants within 100 kB of each gene boundary. The pathway PRSs were normalized to the number of non-zero weighted variants present in that pathway. Logistic regression analyses were performed to test the predictive power of each pathway PRS for POAG status, with age, sex, and the top 10 genotype principal components included as covariates. The pathway PRSs with greatest percent variance explained across EUR and AFR cohorts included TGF-beta signaling (0.57-0.86%), adipogenesis (0.43-0.68%), and angiogenesis (0.25-0.52%) ($p < 0.001$ for all). In summary, this comprehensive pathway-wide approach has identified biological pathways with highest relevance to POAG disease risk. Ongoing work will test the association of these pathway PRSs with specific clinical features. Ultimately, a better understanding of the specific biological pathways driving genetic risk in any given individual may enable personalized approaches to disease diagnosis, prognostication and therapy.

## POSTER 36

# LOOKING BEYOND THE RETINA: COULD OPTIC NERVE GEOMETRY AND COMPOSITE ORBIT FEATURES INFLUENCE GLAUCOMATOUS NEURODEGENERATION?

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Optic nerve traction may contribute to neurodegeneration, particularly in glaucoma. This traction could be influenced by orbital structural characteristics. However, prior studies on optic nerve tortuosity (ONT) and globe geometry differences between glaucoma and controls have been limited in size ($n < 30$) and have not explored relationship with retinal nerve fiber layer thickness (RNFLT) (10.1097/IJG.0000000000001270 10.1167/iovs.17-22093). Leveraging 17940 pairs of 3D T1 magnetic resonance images (MRI) and macular optical coherence tomography (OCT) scans from the UK Biobank (ID 76442), including 370 glaucomatous eyes, we examined associations between orbit structures and macular RNFLT. We developed algorithms to segment MRIs, and extract ONT, globe proptosis (GP), axial length (AL), and a novel composite marker of globe size and position: interzygomatic line-to-posterior pole distance (ILPPD). RNFLT was measured from OCTs and corrected for ocular magnification. RNFLT showed stronger linear correlations with ILPPD than GP ($p < 0.01$). While AL had more correlation with RNFLT, mutual information analyses revealed that ILPPD had a stronger nonlinear relationship with RNFLT than AL. These suggest that the interplay of globe size and position, rather than either alone, may influence neurodegeneration. Additionally, straighter optic nerves and shorter ILPP distances correlated with low RNFLT ($r = 0.065$, $p < 0.001$, and $r = 0.206$, $p < 0.001$, respectively). These relationships were more pronounced in glaucomatous eyes compared to age-sex-matched healthy controls ($p < 0.05$). Our findings suggest that specific orbit structural characteristics could be associated with greater axonal vulnerability. Perhaps, eye movements are restricted by reduced redundancy in straighter nerves and/ or shorter ILPPD indicating an anterior globe-nerve junction or greater proptosis. Our findings suggest that future research should look beyond the retina to investigate orbit structures, particularly how ONT and ILPPD relate to neurodegeneration in the context of eye movement and gaze directions.

## POSTER 37

# VISUAL AND COGNITIVE FUNCTIONAL ABNORMALITIES IN FEMALE 5XFAD MICE WITH ALZHEIMER'S PATHOLOGY ARE WORSENED BY OPTIC NERVE DAMAGE

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Glaucoma and Alzheimer's disease (AD) often present concurrently in elderly patients. To determine if comorbidity worsens functional outcomes, 3mth-old 5XFAD mice of both sexes were subject to either unilateral optic nerve crush (ONC, n = 35) or unilateral intracameral microbead injection-induced ocular hypertension (MB, n = 41), with mice receiving unilateral sham surgery (n = 35) or saline injection (n = 35) as their respective controls. Additional controls included wildtype (WT) mice that underwent the same procedures (ONC n = 29, MB n = 26, sham n = 26, saline n = 28) and naïve mice (5XFAD n = 34, WT n = 24). Intraocular pressure (IOP) was measured weekly in MB-injected mice and their controls from 3-9mths of age, and OCT imaging, optomotor response (OMR) and visual evoked potential (VEP) testing, were conducted at 3- and 9mths of age. Memory was assessed by cued fear conditioning at 9mths. Between-group comparisons used ANOVA with Tukey's post-test, $p < 0.05$ considered significant.

Naïve, 9mth-old female 5XFAD mice had supranormal VEP amplitudes relative to WT littermate controls ($p = 0.0128$) but lower visual acuity on OMR, indicating inherent visual dysfunction. OMRs and VEP amplitudes were largely extinguished ($p \leq 0.01$), while ERG b-wave amplitudes were preserved, in ONC eyes compared to controls across all sexes and genotypes at 9mths-old. Although similarly reduced relative to controls, these metrics were more variable in MB-injected eyes. Female 5XFAD mice exhibited relative freezing deficits during cued fear conditioning compared to WT females ($p = 0.0192$). Freezing behavior also differed significantly between female 5XFAD ONC mice and female 5XFAD sham controls ($p = 0.0214$,) but significant differences in freezing behavior were not seen in other groups. Cognitive function was reduced in female 5XFAD MB-injected mice relative to other groups, though this did not reach statistical significance (ANOVA $p = 0.0548$).

Females have inherent visual functional deficits and enhanced susceptibility to the effects of optic nerve damage on subsequent decline in visual and cognitive function in this model of AD.

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## POSTER 38

## FUNCTIONAL ASSESSMENT OF THE MAPK PATHWAY IN AXONAL REGENERATION

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Using a recently developed optic nerve crush model where axonal degeneration and regeneration are assessed in live Xenopus laevis tadpoles, the Marsh-Armstrong lab has previously reported that the MAP kinase gene Map3k12 (aka Duel Leucine Zipper Kinase, or Dlk) is necessary for retinal ganglion cells (RGCs) both to regenerate their axons and phosphorylate Jun soon after the axon injury, and yet Jun itself is not necessary for the axonal regeneration. To understand this further, we are carrying out a systematic dissection of the MAPK pathway both genetically through CRISPR-Cas9 interference as well as pharmacologically. The knockdown of two genes believed to be downstream of Dlk and upstream of Jun phosphorylation in the axon injury response, Map2k7 and Jnk1, partially reproduce Dlk loss of function and the Dlk inhibitor, GNE-3511, fully phenocopies the Dlk knockout. A pharmacological inhibitor of Map4k, PF-06260933, which in mice produces the same neuroprotection as Dlk inhibition but without an associated inhibition of axonal regeneration, in Xenopus laevis tadpoles has no measurable effect on axonal regeneration, but instead by itself produces axonal degeneration in the absence of optic nerve crush. Overall, these studies suggest that the Dlk pathway acts differently in frogs and mice in regulating axonal injury. Given the importance of this pathway in controlling the response of RGCs to axonal injury, further studies of this pathway are underway with the hopes of determining how it is that frog RGCs regenerate their axons after injury, with the ultimate goal of learning how replicate the same in mice and humans.

## POSTER 39

# THE EFFECT OF THE HIGH-FLOW-PIEZO1-ITGA9 AXIS ON SCHLEMM'S CANAL ENDOTHELIAL CELL PROLIFERATION

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Schlemm's canal (SC), a hybrid vasculature of lymphatic and venous endothelial cells, regulates aqueous humor outflow, and its narrowing leads to elevated intraocular pressure (IOP) and glaucoma. We previously reported that conditional knockout (CKO) of Itga9 results in SC narrowing and IOP elevation (Kiyota N et al., ARVO 2024). ITGA9, known to interact with the extracellular matrix, has been implicated in the proliferation of lymphatic endothelial cells (LECs). We also reported that PIEZO1, a mechanosensitive ion channel activated by flow, upregulates *Itga*9 expression in cultured LECs (Du J et al., JCI 2024). Given the segmental flow pattern in SC, we hypothesized the existence of a High-flow-PIEZO1-ITGA9-endothelial cell proliferation axis involved in SC development and IOP homeostasis. To test this, Piezo1$^{fl/fl}$; Rosa26-rtTA;TetO-Cre mice were administered doxycycline from E19.5 to induce Piezo1 CKO. IOP was measured at 10 weeks using rebound tonometry. SC area was assessed by PECAM1 immunostaining and confocal microscopy. Segmental flow was visualized by FluoTracer™ injection into the anterior chamber of wild-type and *Itga*9 CKO mice, and signal intensity was quantified using ImageJ. SC endothelial cell proliferation was evaluated by EdU incorporation followed by Click-iT and ERG staining. Piezo1 CKO mice exhibited significant SC narrowing (0.73 ± 0.03 vs 0.86 ± 0.03 × $10^4$ μm$^2$, P = 0.018) and elevated IOP (12.6 ± 0.4 vs 10.9 ± 0.4 mmHg, p = 0.009), phenocopying the *Itga*9 CKO phenotype we reported. In *Itga*9 CKO mice, FluoTracer™ intensity was significantly reduced compared to wild-type controls (10.66 ± 3.37 vs 16.73 ± 4.35 AU, p = 0.037). Piezo1 CKO mice also showed a significantly lower percentage of EdU/ERG double-positive cells (1.30 ± 0.26 vs 2.15 ± 0.24%, p = 0.029). These findings support a model in which segmental flow activates Piezo1 to induce *Itga*9 expression, promoting SC endothelial cell proliferation critical for SC development and IOP regulation.

## POSTER 40

# HUMAN OCULAR FLUID OUTFLOW ON-CHIP REVEALS TRABECULAR MESHWORK-MEDIATED SCHLEMM'S CANAL ENDOTHELIAL DYSFUNCTION IN STEROID-INDUCED GLAUCOMA

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Glaucoma, a leading cause of blindness, is characterized by elevated intraocular pressure (IOP) due to impaired fluid outflow through the trabecular meshwork (TM) and Schlemm's canal (SC) endothelium. However, how SC and TM coordinate to regulate outflow under healthy and glaucomatous conditions remains unclear. We developed a human ocular fluid outflow-on-chip system, comprising three-dimensional lymphatic-like SC endothelium surrounded by TM cells, enabling interstitial fluid drainage (Lu et al., Nat Cardiovasc Res., accepted). Using this platform, we modeled steroid-induced glaucoma by treating with dexamethasone (DEX, 1 μM), which reduced fluid outflow by 7.3% ($p = 0.0015$) and thickened SC endothelial junctions by 11.7% ($p = 0.0231$). These phenotypes only occurred when both SC and TM were present, indicating that neither cell type alone is sufficient to form outflow resistance in this condition. Treatment with the TGF-β receptor inhibitor SB431542 (10 μM) reversed steroid-induced glaucoma phenotypes in the chip model, achieving 94% recovery of fluid outflow ($p = 0.0358$). In vivo, SB431542 (10 mg/kg/day, intraperitoneally) also restored drainage in a mouse model of steroid-induced glaucoma, with 72% recovery ($p = 0.0053$). We further identified VEGFC as a key regulator of drainage through junctional loosening in endothelium. DEX suppressed VEGFC expression in TM, which was restored by SB431542. Mechanistically, DEX activates ALK5 in TM cells, which downregulates VEGFC, leading to SC endothelial dysfunction and impaired drainage. Pharmacological inhibition and genetic modulation confirmed the role of the ALK5/VEGFC axis in vivo. Our human ocular outflow-on-chip platform bridges in vitro and in vivo models, offering a tool for studying glaucoma mechanisms.

# POSTER SESSION 2
# HANGING OUT WITH MORE BRILLIANT IDEAS

## POSTER 41

# MICRORNA REGULATION OF GLUCOCORTICOID SIGNALLING IN THE TRABECULAR MESHWORK

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The use of glucocorticoids is rapidly increasing in ophthalmology. One major side effect is elevated intraocular pressure (IOP), which can lead to steroid-induced ocular hypertension (SI-OHT) and glaucoma (SIG). Around 40% of the population and almost all primary open angle glaucoma patients are 'steroid responders', showing raised IOP when exposed to glucocorticoids. miRNAs influence glucocorticoid signalling by regulating glucocorticoid receptors and their pathways. miRNA-based therapeutics offer translational benefits compared to other treatment modalities. This study aims to determine the role of differential miRNA expression in glucocorticoid signalling in the trabecular meshwork and develop miRNA-based therapeutics. Bioinformatic modelling and literature identified specific miRNAs involved in glucocorticoid signalling. miR-18a-5p and miR-124-3p were selected for further study due to regulation of glucocorticoid receptor (NR3C1). Normal trabecular meshwork cell line (NTM5) and a glaucomatous cell line (GTM3) were treated with 100nM of dexamethasone for 24hr, and miR-124-3p and miR-18a-5p expression assessed with RT-qPCR. Key gene targets were profiled, including NR3C1. In untreated NTM5 cells the level of miR-124-3p and miR-18a-5p were similar but dexamethasone treatment suppressed the expression of both miRNAs in only the GTM3 cell line. NTM5 and GTM3 were transfected with miR-124-3p and miR-18a-5p mimics (25nM for 48hr) and treated with dexamethasone for 24hr followed by RNA extraction. The expression of NR3C1, components of the glucocorticoid signalling pathway (FKBP5 and TSC22D3) and downstream effectors were measured using RT-qPCR and Western blotting. miRNA-based therapeutics are an attractive approach to target glucocorticoid induced molecular pathology in the trabecular meshwork. This work aims to understand the interplay between miRNAs and glucocorticoids in the trabecular meshwork and is a step towards developing miRNA-based therapeutics for SI-OHT and SIG.

Funder: Fight for Sight (UK)

## POSTER 42

# PIEZO1-MEDIATED MECHANOTRANSDUCTION PROMOTES RETINAL GANGLION CELL NEUROPROTECTION IN GLAUCOMA

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Although it is well established that elevated intraocular pressure (IOP) is a major risk factor for glaucoma, the role of mechanical signaling in retinal ganglion cell (RGC) degeneration remains poorly understood. We have previously identified rare, coding variants in the mechanosensitive ion channel PIEZO1 that were associated with lower glaucoma risk. Here, in a mouse microbead model of glaucoma, we found that PIEZO1 expression is upregulated in RGCs early after IOP elevation. Activating PIEZO1 through treatment with the chemical agonist Yoda1 or RGC-specific overexpression protects RGCs from degeneration and preserves visual function, despite elevated IOP. Conversely, RGC-specific PIEZO1 knockout decreases RGC survival following IOP elevation. PIEZO1-mediated $Ca^{2+}$ influx promotes activation of $Ca^{2+}$/calmodulin-dependent protein kinase II (CaMKII), which is a key regulator of RGC survival. Inhibition of CaMKII abrogates PIEZO1-mediated RGC neuroprotection. Thus, these data define PIEZO1-CaMKII as a neuroprotective signaling pathway in RGCs, and shift the paradigm for understanding how mechanotransduction can be targeted to treat glaucoma.

## POSTER 43

# HUMAN IPSC-DERIVED MITOVESICLES ENHANCE RETINAL GANGLION CELL RESCUE AND PROMOTE AXONAL ARBORIZATION: A NOVEL NEUROPROTECTIVE STRATEGY FOR OPTIC NEUROPATHIES

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This study aimed to harness the neuroprotective potential of mitovesicle-enriched small extracellular vesicles (mito-sEVs) to elucidate underlying mechanisms of their effect on retinal ganglion cells (RGCs), to develop more effective and clinically viable therapeutic interventions. In pursuit of this goal, we purified mito-sEVs from an iPSC line using buoyant density fractionation and compared their neuroprotective effects in a primary mixed retinal cell culture. We analyzed transcriptomic profile of mito-sEVs vs UNF-sEVs to identify differentially expressed genes and distinct pathway enrichments associated with their neuroprotective potential. Among the 30 sEV fractions, fractions 16 to 20-designated as mito-sEVs, exhibited the highest vesicle yield ($1.83x10^{12}$ vesicles/100mL condition medium) and demonstrated markedly elevated PDHE1α expression, a canonical mitovesicle marker, relative to other fractions. Mito-sEVs elicited the highest RGC survival compared to untreated controls (~200%, $p < 0.0001$) and other fractions. Compared to UNF-sEVs, mito-sEVs significantly enhanced RGC morphometric parameters, including a higher proportion of surviving RGCs with axons and neurites (~70%), RGCs lacking axons (~49%), and those bearing neurites exclusive of axons (~59%). Furthermore, mito-sEVs treatment markedly increased the number of primary (~68%), secondary (~53%), and tertiary (~60%) neurites, as well as axonal arborization (~75%), indicating robust promotion of neurite outgrowth and structural complexity. Transcriptomic analysis revealed 32 uniquely upregulated protein-coding mRNAs in mito-sEVs versus UNF-sEVs, which were significantly enriched in energy metabolism pathways—especially oxidative phosphorylation and ATP biosynthesis—implicating their role in augmenting RGC survival and neurite growth. Thus, mito-sEVs can offer a promising neuroprotective approach for treating optic neuropathies by improving RGC survival and promoting axonal arborization through energy-driven pathways.

## POSTER 44

# NEURITIN MAINTAINS RETINAL GANGLION CELL FUNCTION AND VISUAL PATHWAY CONNECTIVITY

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Retinal ganglion cell (RGC) degeneration is a hallmark of glaucoma. Neuritin (Nrn1), a protein associated with AMPA receptor complexes, is preferentially expressed in RGCs and implicated in synaptic plasticity and axonal growth, yet its role in visual pathways remains unclear. We investigated the impact of Nrn1 loss on RGC function, axon projection, and visual behavior. Pattern electroretinography revealed reduced P1–N2 amplitude in Nrn1 knockout (KO) mice compared to wild-type (WT), indicating impaired RGC function. RNAscope and immunostaining confirmed RGC-specific expression of Nrn1. Despite this dysfunction, optomotor reflex testing showed no difference in spatial frequency or contrast sensitivity between KO and WT mice. This aligns with preserved axon projections to accessory optic system (AOS) targets - including the medial terminal nucleus and nucleus of the optic tract - as revealed by cholera toxin B-based anterograde tracing. While the dorsal lateral geniculate nucleus (dLGN) showed reduced signal intensity in KO mice, its axonal coverage remained intact. These preserved subcortical circuits likely support reflexive visual behavior. In contrast, KO mice exhibited significantly reduced axon projections to non-AOS targets, including the superior colliculus (SC), olivary pretectal nucleus, ventral LGN, and suprachiasmatic nucleus (SCN), supporting selective disruption of long-range visual pathways. Notably, these deficits occurred despite minimal reported Nrn1 expression in SC and SCN, suggesting that axonal abnormalities arise from RGCs rather than target-driven degeneration. RNA sequencing identified downregulation of genes involved in postsynaptic density and Notch signaling. Finally, intravitreal delivery of Nrn1 protein with its binding partner Olfactomedin1 significantly enhanced RGC survival after optic nerve crush. These findings demonstrate that Nrn1 is critical for RGC integrity and connectivity and may represent a therapeutic target in glaucomatous neurodegeneration.

## POSTER 45

# NEUROPROTECTION OF RETINAL GANGLION CELLS IN EXPERIMENTAL GLAUCOMA USING A NOVEL GENE THERAPY CONSTRUCT AAV2-HSYN1-TRKB-2A-MBDNF IN ADULT AND OLD MICE

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The efficacy of boosting brain derived neurotrophic factor (BDNF) expression via gene therapy with recombinant adeno-associated viral (AAV) vectors to enhance retinal ganglion cell (RGC) survival in glaucoma is limited by downregulation of its receptor, TrkB. We investigated whether enhancing expression of both mature BDNF (mBDNF) and TrkB, under control of the hSYN1 promotor, provided neuroprotection in experimental glaucoma (EG) in adult and old mice.

Thy1-YFP-H and C57BL/6 adult (3-month-old) and old (18-month-old) mice received an intravitreal injection of AAV2 TrkB-2A-mBDNF. After 3 weeks, individual retinal extracts were analysed by western blots. mBDNF, TrkB and anti-apoptotic markers upregulated by mBDNF, phosphorylated-Akt (p-Akt) and phosphorylated-ERK (p-ERK), were measured in experimental (Ex) and contralateral control (Co) eyes. To assess neuroprotection, 2-year-old mice received intravitreal AAV2 TrkB-2A-mBDNF (treated) or hSYN1-mCherry (sham-treated) 3 weeks before EG induction with the hydrogel model and followed for 56 days. IOP was monitored weekly and RGC density estimated with Brn3A immunohistochemistry.

There was significant mBDNF upregulation in Ex compared with Co eyes, with a 16-fold and 84-increase in mBDNF/BDNF ratio in adult and old mice, respectively. There was a 12-fold and 14-fold increase in the ratio of TrkB/β-actin in Ex compared to Co eyes in adult and old mice, respectively. pERK/ERK ratio showed a 1.5-fold and 1.2-fold increase in the adult and old Ex eyes, respectively, compared to their Co eyes. pAkt/Akt ratio showed a 1.8-fold and 1.7-fold increase in the adult and old Ex eyes, respectively, compared to their Co eyes.

In the old mice injected with AAV2 pre-EG, there was a significantly higher RGC density in treated vs. sham-treated eyes (10% vs. 20% loss of RGCs), compared to untreated Co eyes.

This novel construct, under the hSYN1 promotor, provided strong neuroprotective environment for RGCs, and enhanced RGC survival in 2-year-old mice injected prior to disease onset.

## POSTER 46

# ADDITIVE EFFECT OF STANNIOCALCIN-1 WITH TIMOLOL BUT NOT LATANOPROST IN A MOUSE MODEL OF OCULAR HYPERTENSION

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Stanniocalcin-1 (STC-1) is a secreted, multifunctional hormone required for prostaglandin analog-mediated intraocular pressure (IOP) lowering. Our laboratory has found that STC-1 has standalone ocular hypotensive properties in ex vivo human eyes as well as in both normotensive and ocular hypertensive mice. Unlike prostaglandin analogues, STC-1 does not require the prostaglandin F (FP) receptor for IOP reduction. To evaluate STC-1 as a potential combination drug, we tested whether STC-1 would function in an additive manner with timolol or latanoprost to lower IOP in a steroid-induced mouse model of ocular hypertension. To determine the optimal STC-1 concentration, normotensive C57BL/6J mice (n = 5) were treated once daily for four days with six concentrations of STC-1 (0.000005 to 0.5 mg/ml). IOP was measured at 1, 4, and 23 hours using rebound tonometry. IOP reduction was observed in a dose-dependent manner with the plateau of IOP reduction being 0.05 mg/mL. For combination studies, ocular hypertension was induced via weekly periocular dexamethasone injection (20 µL, 10 mg/ml) for 6 weeks. After successful IOP increase, mice (n = 30) were randomized to timolol (0.5%), latanoprost (0.005%), or no treatment as a control (n = 10 each). One eye initially received monotherapy (days 1-4), followed by combination therapy with STC-1 (days 5–8), and finally STC-1 alone (days 9-12). Combination treatment with timolol and STC-1 reduced IOP by 38% (-7.29 mmHg from 19.33 mmHg, p = 0.01), significantly more than timolol (3.43 mmHg, p = 0.01) or STC-1 (4.43 mmHg, p = 0.11) alone. In contrast, no additive effect was seen with latanoprost, as the reduction with STC-1 combination (4.51 mmHg, p = 0.038) was similar to latanoprost (3.98 mmHg, p = 0.118) or STC-1 (4.93 mmHg, p = 0.473) alone. Since STC-1 was additive with timolol but not latanoprost for IOP reduction, and STC-1 lowers IOP downstream of latanoprost, we hypothesize that latanoprost and STC-1 have mechanistic overlap.

## POSTER 47

# CHARACTERIZATION OF MYOCILIN'S COILED COIL DOMAIN AND INTRINSICALLY DISORDER LINKER REGION USING NMR

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Mutations to Myocilin lead to a subset of primary open-angle glaucoma (POAG), which is itself the most common glaucoma subtype. The irreversible blindness resulting from myocilin-associated POAG is caused by a toxic gain of function in the trabecular meshwork region of the eye. Mutant myocilin aggregation remains localized to the endoplasmic reticulum (ER), which leads to trabecular meshwork cell death and subsequent increase of intraocular pressure, the causal risk factor for glaucoma. The large majority in pathogenic mutations are in the olfactomedin (OLF) domain, with the other domains being the N-terminal coiled-coil (CC), the leucine zipper (LZ), and a 60-residue linker region predicted to be intrinsically disordered (IDR) that connects OLF to LZ. While the lab has solved crystal structures of OLF and LZ, no structure of full-length currently exists. While the lab has previously produced an envelope of the CC domain by small angle x-ray scattering, no atomistic structure of the CC domain exists. In addition, little is currently known about the intrinsically disordered linker domain with respect to its contribution to the structure of full-length myocilin and its biological function. The aim of this project is to use biomolecular NMR to structurally characterize both the CC and linker domains to provide insight into the molecular structure of full-length myocilin.

## POSTER 48

# STRUCTURAL CHARACTERIZATION OF PSEUDOEXFOLIATION GLAUCOMA-ASSOCIATED LOXL1: APPROACHES, CHALLENGES, AND PROGRESS

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Pseudoexfoliation syndrome (XFS) affects ~70 million people globally. Approximately one-third of XFS cases will develop pseudoexfoliation glaucoma (XFG), making XFG the most common form of secondary open-angle glaucoma. XFG is characterized by accumulation of non-degradable exfoliation material (XFM) in the anterior chamber, leading to reduced aqueous humor outflow and severely elevated intraocular pressure. Current treatments fail to address the root cause of XFG, the formation of XFM. XFM contains many extracellular matrix proteins, including elastin and elastin cross-linking protein LOXL1. Coding variants in LOXL1 have strong genetic association to XFG. The location of these variants near the tropoelastin-interacting domain and an activating cleavage site strongly suggest functional impact. A major hurdle to understanding the functional impact of these variants is the current limited biochemical characterization of wild-type LOXL1. Structural modelling software yields low confidence predictions for LOXL1, particularly for the variant-containing N-terminal domain, which is predicted to be highly disordered. This project has sought to purify wild-type LOXL1 and its variants for biochemical and structural characterization. Full-length LOXL1 and its Cricetulus griseus homolog LOX were purified from ExpiCHO spent media and cell lysate. Proteolytic cleavage in spent media (LOXL1), chaperone binding in cell lysate (LOXL1), and low protein yields (LOX) posed obstacles to the high concentrations of pure protein required for structural characterization. As a result, we are now taking a divide-and-rebuild approach in which the disordered N-terminal domain and the catalytic C-terminal domain are expressed separately in ExpiCHO or E. coli. Challenges to stability and purity have been encountered for both constructs, but numerous troubleshooting avenues are being pursued. In conclusion, high demands for concentration, stability, and purity for protein structural characterization present challenges. However, LOXL1 structural characterization will be pivotal for understanding XFG etiology and developing the first treatments that address the unique cause of this blinding disease.

## POSTER 49

# GENETIC MODULATION OF TRPV4 CHANNELS IN RETINAL GANGLION CELLS PROFOUNDLY AFFECTS GLIAL ACTIVITY AND NEURODEGENERATION IN MOUSE GLAUCOMA

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IOP lowering slows the progressive loss of retinal ganglion cells (RGCs), indicating that glaucoma is a disease of pathological pressure sensing in these projection neurons. Ocular hypertension (OHT) induces dendritic/synaptic pruning, changes in gene expression and axonal transport long before visible nerve fiber thinning or optic nerve head excavation. This suggests that sensory transduction in RGCs is compartmentalized with the identity, properties and function of the mechanosensory molecules remaining largely unknown. Here, we investigate the expression of TRPV4, a nonselective cation channel that mediates the RGC sensitivity to swelling and mechanical stress.

Given the potential complications associated with antibody labeling, TRPV4 expression was tracked by combining RGC-specific TRPV4 expression with animal models of OHT. AAV2 was utilized to deliver TRPV4-eGFP driven by CMV-synuclein-γ (Scng) promoter in mice. TRPV4-EGFP expression was tracked in vivo using the Micron IV retinal imaging system and in fixed tissue isolated at week 6 post-transfection. TRPV4 expression was tracked in alpha-RGCs and ipRGCs of normotensive and hypertensive animals. The iridocorneal angle was obstructed through microbead (MB) injection.

AAV2-transfected retinas showed elevated Trpv4 mRNA content. TRPV4-EGFP was observed in RGC axons and neurites, and absent from amacrine cells and bipolar cells. Prominent TRPV4-EGFP was observed in RGC axons, with fluorescence in the proximal ONH more conspicuous compared to the distal ONH. ONH Trpv4 mRNA was several-fold higher compared to the retina. Gfap mRNA levels were increased in the retina of transfected animals. MB microinjections elevated IOP, induced RGC death and altered TRPV4 expression.

Our data show that TRPV4 expression is compartmentalized in dendrites, somata, and axons, indicating the potential link between OHT stress and early changes in local architecture and signaling in mammalian RGCs. Laminar remodeling may represent a late-stage event that is preceded by parallel mechanosensor-dependent remodeling, presumably involving changes in calcium homeostasis and calcium-dependent signaling.

## POSTER 50

# UNRAVELING RIG-I–MEDIATED GLAUCOMA MECHANISMS AND THERAPEUTIC AVENUES IN A MOUSE MODEL OF SINGLETON-MERTEN SYNDROME

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Singleton-Merten Syndrome (SGMRT) is a rare immunogenetic disorder caused by dominantly inherited gain-of-function variants in RIG-I/DDX58. Patients exhibit variable systemic manifestations, including juvenile open-angle glaucoma (JOAG), psoriasiform rash, vascular and joint calcifications, and tendon rupture. While previous studies have reported interferon (IFN) pathway activation in patient tissues, the mechanisms linking RIG-I variants to glaucoma remain poorly understood.

To address this gap, we generated a CRISPR/Cas9 knock-in (KI) mouse model carrying the p.E511V variant (orthologous to p.E510V found in two families). KI mice exhibited hallmark features of glaucoma, including elevated intraocular pressure (IOP), reduced ganglion cell complex thickness, reduced retinal ganglion cell number, and decreased Schlemm's canal area. Expression levels of Rigi and IFN stimulated genes (ISG), Isg15, Mx1, and Oas2, were found increased in limbal strips, indicating a strong Type I IFN response. Homozygous mice also showed reduced body weight and abnormal kidney morphology, recapitulating SGMRT systemic features.

Single-cell RNA sequencing of limbal tissues revealed upregulated IFN signaling across multiple cell types, including trabecular meshwork, endothelial, and immune clusters. Pathway analysis indicated altered cytoskeletal and motility pathways in trabecular meshwork cells and impaired cell proliferation with decreased cell numbers in the endothelial cluster.

To evaluate potential therapies, Rigi KI/KI mice were treated with an IFN receptor (IFNAR) blocking antibody, which partially rescued IOP and systemic phenotypes. IFNAR blockade normalized Rigi expression in ocular tissues, kidneys and spleen and reduced ISG levels, with complete rescue observed in kidney and spleen.

Our findings establish a direct link between RIG-I gain-of-function variants and the development of glaucoma, providing insights into the molecular mechanisms underlying SGMRT. Moreover, they support the modulation of the IFN pathway as a novel therapeutic approach.

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## POSTER 51

# DEVELOPMENT OF TARGETED OPTIC NERVE HEAD DELIVERY SYSTEMS FOR TREATMENT OF OPTIC NEUROPATHIES

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**Background:** Glaucoma is an optic neuropathy where the primary site of injury is at the optic nerve head. Current treatments are focused on reducing intraocular pressure but do not directly address the diseased optic nerve head. Treatment paradigms are currently limited in their ability to directly target the optic nerve head, and this may be due to limited drug delivery systems targeting that tissue. Here we describe a novel targeted approach to the optic nerve head, called SupraChoroidal-to-Optic-NervE delivery (SCONE).

**Methods:** Right eyes of albino New Zealand White rabbits (N = 25) were prepared for injection. A microneedle was used to create a sclerotomy to enable access to the suprachoroidal space. A catheter was introduced into the suprachoroidal space and directed to the optic nerve head under direct visualization. 5 µL of India ink solution was injected into the optic nerve head. Fundus photography and optical coherence tomography were performed pre-injection, immediately after, at 1 week, and thereafter. Parameters such as microneedle gauge, catheter gauge, and catheter bevel angle were varied. Success of injection and complications were tabulated. Visual evoked potentials (VEP) were performed. Similar optimization of parameters were performed in ex vivo pig eyes.

**Results:** Up to 88% success rate was achieved with an optimized SCONE delivery approach in rabbits in vivo. Microneedle gauge of 27G, catheter gauge of 31G, catheter bevel angle of 45 degrees, and no catheter guard were selected as optimal. In some cases, OCT showed a change in optic nerve head concavity immediately after the injection. There was no significant difference between SCONE treated eyes and contralateral naïve control eyes. Similar success was achieved in ex vivo pig eyes.

**Conclusion:** SCONE may enable targeted drug delivery to the optic nerve head. SCONE-mediated delivery of neuroprotective and neuroregenerative therapies may be useful in the development of paradigm shifting treatments for optic neuropathies.

## POSTER 52

# THE ROLE OF TREM FAMILY GENES IN AN ACUTE INDUCIBLE MODEL OF OCULAR HYPERTENSION

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Targeting microglial states has become an attractive therapeutic objective due to their early involvement in the pathogenesis of glaucoma and other neurodegenerative diseases. Our previous work demonstrated that global reduction of microglia increases susceptibility to optic nerve damage in an ocular hypertension (OHT) model. In recent years, alterations in microglial states have been correlated with later-stage glaucoma severity; however, their specific roles remain unclear.

In the brain, disease-associated microglia (DAM) depend on TREM2 signaling and are thought to be neuroprotective, though their role in glaucoma has not yet been investigated. We hypothesize that genetic ablation of Trem2, which prevents DAM formation, will differentially affect glaucomatous optic nerve damage in an inducible OHT model compared to global microglial depletion. In parallel, TREM1 signaling has also been implicated as critical for optic nerve health in OHT. Therefore, we have additionally genetically ablated Trem1 and assessed optic nerve integrity in the same inducible model.

Using a photopolymerizable biomatrix, we induced a mild increase in IOP in B6-WT, TREM2-KO, and TREM1-KO mice. Longitudinal IOP measurements were taken across genotypes and between experimental and control treatment groups. One month following OHT induction, optic nerves were assessed for glaucomatous damage using p-phenylenediamine staining.

Although subtle, knockout of TREM2 and TREM1 appears to confer some protection compared to WT control mice. To further validate our findings, ongoing studies are evaluating retinal ganglion cell soma and axon integrity. Additionally, we are using immunohistochemistry to explore the relationship between microglia, peripheral immune cells (macrophages and monocytes), and Trem family genes during glaucomatous pathogenesis.

The long-term goal is to elucidate the mechanisms by which microglia and peripheral immune cells contribute to neuroprotection during glaucomatous neurodegeneration.

## POSTER 53

# BIOMECHANICAL FORCES AND PIEZO1 ACTIVATION ALTER OPTIC NERVE HEAD ASTROCYTE MORPHOLOGY AND METABOLIC FUNCTION

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In glaucoma, elevated intraocular pressure coincides with increased tensile biomechanical strain on the optic nerve head (ONH). Local astrocytes are prime supportive cells to neighboring neurons, and respond to pressure elevation by remodeling their actin cytoskeleton and altering metabolic support. A key astrocytic sensor of biomechanical strain is the mechanosensitive channel, Piezo1. Here, we use our 3D hydrogel system to investigate the role of Piezo1 in strain-mediated morphologic and metabolic dysregulation. To determine this, mouse ONH astrocyte-encapsulated hydrogels (N = 3) were treated with vehicle control, Piezo1 agonist Yoda1 (10uM), mechano-inhibitor GsMTx4 (500nM), and co-treatment for 24 and 72h. Separately, constructs underwent 0% or 5% tension +/- GsMTx4 for 24h using the FlexCell System. Following all treatments, astrocyte viability remained unchanged as measured by MTS/Live-Dead assays. Both Yoda1-treated and biomechanically strained groups showed altered fiber alignment and reduced F-actin area ($p < 0.05$), decreased process complexity and degree of branching ($p < 0.05$), and increased nuclear volume ($p < 0.005$); GsMTx4 co-treatment rescued branching and nuclear features. Moreover, strained astrocytes demonstrated varied metabolic response by altering C3/MCT4 mRNA levels ($p < 0.0001$) and increasing lactate release ($p < 0.005$). Given the changes in astrocyte morphology with Piezo1 activation and strain, we next asked whether the hydrogel constructs undergo global matrix alterations. Rheology measures of hydrogel stiffness showed no change with strain but a softening with GsMTx4. Yoda1 treatment did not impact hydrogel contraction, but co-treatment with GsMTx4 decreased contraction. Together, our data implicate Piezo1 in ONH astrocyte response to glaucomatous tensile strain and support therapeutic potential for GsMTx4. Future studies will investigate the role of Piezo1 in strain-induced morphologic and metabolic changes via cell-specific knockdown and live-cell imaging.

## POSTER 54

## OPTIMIZATION OF DATA SAMPLING FOR IOP AND CSFP TELEMETRY

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Cerebrospinal fluid pressure (CSFP) plays a significant role in modulating IOP's impact on the optic nerve head (ONH). Both IOP and CSFP fluctuations are implicated in the development and progression of glaucoma. Hence, this study aimed to determine the minimum data sampling frequency and duty cycle necessary to capture accurate transient IOP and CSFP pressure fluctuation data. Data were collected continuously at 250 Hz for 24 hours in 3 non-human primates implanted with the TSE-Systems Stellar total implant system. IOP data were collected via a fluid-filled silicone tube implanted in the anterior chamber coupled to an extra-orbital pressure transducer. Intracranial pressure data were collected via a pressure transducer implanted in the brain parenchyma level with the ONH, as a surrogate for retrobulbar CSFP. Data were subsampled via a custom MATLAB program that simulated: 1) duty cycles of 80-5% sampling time per 2-min interval, and 2) data sampling frequencies of 200-25 Hz. Data were subjected to a dual-band finite impulse response filter to detect transient fluctuations. Transient IOP and CSFP fluctuations were counted, quantified, extrapolated, and then averaged hourly over 24 hours. Percentage errors were plotted to determine what ranges of duty cycle and sampling frequency produced transient IOP and CSFP data within ± 5% error when compared to continuous 250 Hz data. On average, transient IOP and CSFP fluctuations $^{3}$2.5 mmHg in magnitude occurred ~7000 and ~5300 times/hour, respectively. Duty cycles $^{3}$10% can accurately detect transient IOP and CSFP fluctuation frequency and magnitude with < 5% error. Transient IOP and CSFP fluctuation magnitude and frequency can be detected with < 5% error at ≥ 50 Hz and ≥ 150 Hz data sampling, respectively. Device companies can use this information to design telemetry systems that accurately acquire dynamic IOP and CSFP data to further our understanding of glaucoma.

## POSTER 55

# VALIDATION OF THE EYEMATE SUPRACHOROIDAL SENSOR FOR TELEMETRIC MEASUREMENT OF INTRAOCULAR PRESSURE IN NORMAL EX VIVO EQUINE AND CANINE GLOBES - PRELIMINARY RESULTS

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**Purpose:** To determine the accuracy of the EYEMATE suprachoroidal sensor (EYEMATE-SC; Implandata) for telemetric measurement intraocular pressure (IOP) in horses and dogs using ex vivo equine and canine globes. The EYEMATE-SC is currently approved for clinical use in humans within Europe.

**Methods:** The EYEMATE-SC sensor (7.8 mm x 3.8 mm x 1 mm) was implanted in the suprachoroidal space of two freshly enucleated normal equine eyes and four freshly enucleated normal canine eyes. The anterior chambers were cannulated and connected to a reservoir of Plasma-Lyte A and a manometer. Starting at a manometric IOP of 5 mmHg, the pressure was progressively increased to 80 mmHg by raising the reservoir. Triplicate IOP measurements were taken with the EYEMATE-SC using a portable reading device for telemetric pressure transmission via a radiofrequency band. These measurements were compared to manometric pressure by linear regression analysis.
**Results:** A strong positive linear regression is observed between EYEMATE-SC and manometry IOPs in both equine and canine eyes (equine: $r^2 = 0.99$, canine: $r^2 = 0.99$). The EYEMATE-SC was unable to measure pressures ≥ 70 mmHg in either species.

**Conclusions:** Measuring equine and canine IOPs from the suprachoroidal space using the EYEMATE-SC provided accurate results over an extensive range of pressures in ex vivo globes. This telemetric sensor can assist with long-term, frequent tonometry by pet owners and clinicians. Although the sensor could not detect pressures above 70 mmHg, this flaw was not considered clinically relevant.

## POSTER 56

# VITREOUS INFLAMMATION AS A DANGER SIGNAL FOR RETINAL GANGLION CELL LOSS FOLLOWING OPTIC NERVE INJURY

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This study aims to investigate the identity and function of the vitreous hyperreflective foci (VHRFs) detected by visible light optical coherence tomography (vis-OCT) right after the acute optic nerve crush (ONC) injury. Adult wild-type C57BL/6 mice and transgenic CX3CR1GFP/GFP mice were used for in vivo vis-OCT imaging, followed by ex vivo confocal imaging. First, vis-OCT tracking of the same mouse eyes was performed before and at 6, 12 hours, 1, 3, 6, and 12 days post-ONC. The VHRFs appeared at 12 hours post-ONC, while no significant RGC loss was detected. At one day post-ONC, the VHRFs remained elevated, with 9.56 % of RGC loss observed. After vis-OCT imaging, mouse eyes were prepared for whole-eye sagittal sectioning, followed by immunostaining and confocal imaging to characterize the VHRFs. Our data showed that the VHRFs are round-shaped CX3CR1-GFP$^{+}$ Iba1$^{+}$ CD68$^{+}$ cells, representing activated amoeboid microglia in the vitreous and retina at 1-day post-ONC. Next, we examined the neuroprotective effects of Anakinra, an interleukin-1 receptor antagonist, to inhibit microglia-mediated inflammation. Anakinra was intracamerally injected immediately after ONC to inhibit VHRFs. We observed a decreased number of VHRFs at 18 hours and improved RGC survival at 3 days post-ONC. Taken together, our study showed that VHRFs occurred within 1-day post-ONC, preceding significant RGC loss, suggesting they may serve as a “danger signal.” The VHRFs are activated microglia; their inhibition improved RGC survival post-ONC. Our results lay the foundation for early detection and therapeutic intervention in RGC degeneration and vision loss.

## POSTER 57

# CONDITIONAL EXPRESSION OF THE TVA RECEPTOR IN RETINAL GANGLION CELLS PERMITS CELL-SPECIFIC TRANSDUCTION WITH ENVA-PSEUDOTYPED VIRUSES

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Targeting rare, disease-susceptible cell types with specific viral platforms can advance gene delivery and perturbation applications. Retinal ganglion cells (RGCs) comprise ~1% of retinal cells and are vulnerable to degeneration in glaucoma. While adeno-associated viruses (AAVs) offer tropism for RGCs, they lack full specificity and are constrained by limited packaging capacity. This project aims to engineer a mouse model where RGCs selectively express the avian tumor virus receptor A (TVA), permitting efficient and specific infection with EnvA-pseudotyped viruses. Using CRISPR-Cas9, we knocked in a LoxP-STOP-LoxP-TVA-GFP cassette into the ROSA26 locus. Heterozygous founders were confirmed by genotyping and Sanger sequencing, then bred with Vglut2-Cre mice to drive TVA and GFP expression in RGCs. Immunofluorescence on retinal sections from Vglut2-Cre-TVA mice was used to detect TVA, GFP, and RBPMS. Rod-depleted suspensions of Vglut2-Cre-TVA retinal cells were transduced in vitro with EnvA-pseudotyped lentivirus carrying mCherry. In Vglut2-Cre-TVA mice, expression of TVA and GFP predominantly co-localized with RBPMS, indicating specific expression in RGCs, consistent with expected Vglut2-Cre expression. Minor expression was also observed in the inner plexiform layer and inner nuclear layer, likely correlating to a subset of Vglut2-expressing type-5 bipolar cells. *In vitro* culture of retinal cell suspensions following EnvA-lentivirus transduction demonstrated an overlap of GFP and mCherry staining. No mCherry was detected in other cells, confirming specificity. This work demonstrates successful engineering of a Cre-dependent TVA mouse line enabling selective EnvA-lentivirus transduction. Ongoing in vivo studies will map transduction patterns after intravitreal injection of EnvA-lentivirus. This system enables targeted gene delivery to rare retinal cell types using lentiviral vectors with larger payload capacity.

## POSTER 58

# IOP BUT NOT AGE INCREASES LOSS OF OPTIC NERVE AXONS IN MICE WITH A DEFICIENCY IN MACROGLIAL INTERMEDIATE FILAMENTS

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Glial fibrillary acid protein (GFAP) and Vimentin (VIM) are major intermediate filament components of astrocytes and contribute significantly to their biological functions. In human and experimental glaucoma, astrocytes show signs of reactivity, distinct morphological changes and an increased immunoreactivity for GFAP and VIM. Several studies in animal models of glaucoma reported on an association of the reactive phenotype with severe axon loss. In this study we aimed to investigate how a complete deficiency in GFAP and VIM influences retinal ganglion cell (RGC) survival under ocular hypertension and with increasing age.

GFAP and VIM deficiency was confirmed by Western Blot. Ocular morphology was analyzed using semi-thin sections by light microscopy. Ocular hypertension was induced by injection of magnetic microbeads. Intraocular pressure (IOP) was monitored over time. Myelinated ON axons were counted in PPD-stained ON cross-sections, whereas RGC somata were quantified in retinal wholemounts stained against RBPMS.

Deficiency in GFAP and VIM had no observable effects on ocular morphology, axon number or IOP. After 6 weeks of ocular hypertension axon and RGC loss was significantly higher in $GFAP^{-/-}$ $VIM^{-/-}$. Axon and RGC numbers declined with increasing age in all animals. No statistically significant differences were found between $GFAP^{-/-}$ $VIM^{-/-}$ and wildtype animals at 6 and 12 month of age.

Our findings strongly indicate, that macroglial intermediate filaments play a crucial role in the eyes ability to cope with ocular hypertension. It remains to be elucidated if the observed negative effect is caused by biomechanical alterations at the optic nerve head or by altered astrocyte and/or Müller cell homeostatic functions.

## POSTER 59

# SMALL-MOLECULE MITOCHONDRIAL UNCOUPLING THERAPY PROMOTES NEUROPROTECTION AND PRESERVES VISUAL FUNCTION IN EXPERIMENTAL GLAUCOMA

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Ocular hypertension (OHT) impairs mitochondrial function, leading to increased reactive oxygen species (ROS) production and retinal ganglion cell (RGC) death. MP201 is a slow-release prodrug of 2,4-dinitrophenol (DNP), a brain-penetrant mitochondrial uncoupler that counters damage by reducing ROS and calcium ($Ca^{2+}$) overload while promoting cellular repair. Here, we tested whether MP201 improves mitochondrial health and promotes RGC resilience in experimental glaucoma.

The magnetic microbead model was used to induce OHT in adult mice. MP201 (8 mg/kg), was administered daily via oral gavage starting one week after OHT induction. ROS levels were assessed using the superoxide indicator dihydroethidium (DHE), and mitochondrial $Ca^{2+}$ was measured using Rhod2-AM. Mitochondrial volume was evaluated in Thy1-YFP-CFP-Mito mice. Retinal BDNF levels were measured by western blot and immunofluorescence. RGC density was quantified in RBPMS-stained retinas using an unbiased stereological approach. Visual acuity was assessed by measuring the optomotor reflex. Intraocular pressure (IOP) and body weight were measured weekly to monitor adverse effects.

MP201 significantly improved RGC health by: i) reducing ROS production (N = 6/group; Dunnett's ANOVA, *p = 0.022), ii) mitigating $Ca^{2+}$ overload (N = 6/group; *p = 0.014), iii) replenishing axonal mitochondrial volume (N = 6/group; Dunnett's ANOVA, *p = 0.039), and iv) increasing endogenous retinal BDNF levels. Importantly, MP201 promoted RGC survival (97.5% ± 3.8% vs. 81.3% ± 3.7%; N = 6/group; Dunnett's ANOVA, ***p = 0.003) and improved visual acuity (N = 6/group; Dunnett's ANOVA, *p = 0.001) relative to vehicle-treated controls. MP201 did not reduce IOP or caused weight loss.

Our results demonstrate that oral MP201 treatment enhances RGC resilience, promotes neuronal survival, and preserves visual function. This work identifies mild mitochondrial uncoupling as a promising therapeutic strategy to counter oxidative stress and protect against vision loss in glaucoma.

## POSTER 60

# FUNCTIONAL VALIDATION OF FRAGMENT HITS TARGETING THE MYOCILIN OLFACTOMEDIN DOMAIN

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Primary open-angle glaucoma (POAG) is the most common form of glaucoma, affecting over three million individuals in the United States [1]. A key contributor to POAG pathogenesis is the misfolding and aggregation of myocilin, a secreted glycoprotein localized in the trabecular meshwork (TM). A subset of POAG cases has been linked to mutations in the MYOC gene, particularly within its olfactomedin (OLF) domain, causing glaucoma in children and young adults. These mutations promote protein misfolding, endoplasmic reticulum (ER) retention, and subsequent stress and toxicity in TM cells [2]. Notably, despite intense interest and impact on human disease over 20 years, the biological function of myocilin has remained elusive.

To identify novel ligands binding to the OLF domain, a fragment-based drug discovery (FBDD) screen was conducted using the XChem pipeline. This process identified two molecular surfaces on OLF capable of binding several different classes of small molecules. The current objective is to further characterize these hits to narrow down the site for future molecule elaboration, such as assays for that probe for the effect of the identified compounds on OLF thermal stability and on aggregation. In the long term, these studies will lead to novel molecules for use as chemical probes of myocilin function or myocilin-directed therapeutics.

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## POSTER 61

# UNLOCKING NEUROPROTECTION: NEURITIN'S PROMISE FOR GLAUCOMA

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**Purpose:** Glaucoma, a progressive neurodegenerative disease, has traditionally been treated by regulating a patient's intraocular pressure (IOP), yet in many cases, the pathologic deterioration of retinal ganglion cells (RGCs) continues despite lowering the IOP. A neuroprotective strategy proposed for these patients involves leveraging neurotrophic factors to target RGC loss. Previous research has linked RGC degeneration in glaucoma to neurotrophic factor deprivation. Our prior studies have demonstrated that secreted human neuritin1 (NRN1) protects RGCs following axonal injury in acute glaucoma models and ex-vivo human pressurized eyes. We aim to evaluate NRN1's therapeutic effects on derived RGCs from glaucomatous and non-glaucomatous donors.

**Methods:** Human induced pluripotent stem cells (iPSCs) were derived from keratocytes of non-glaucomatous and glaucomatous donors (N = 3) using CytoTune Sendai reprogramming. The cells were differentiated into retinal organoids (ROs) and RGCs. Protein and gene expression assays characterized keratocytes (α-SMA, Keratocan), hiPSCs (TRA-1-60, OCT4, SOX2, NANOG, C-MYC, KLF4), and RGCs (RBPMS, BRN3A). The hiPSC-derived RGCs were transduced with AAVs to overexpress NRN1 (IRES-hNRN1-RFP), provide a scramble control (scrmb-shRNA-eGFP), or silence NRN1 (hNRN1-shRNA-eGFP). We evaluated vector expression, RGC viability, apoptosis, and neurite outgrowth two weeks post-transduction. Additionally, AAV2-transduced RGCs from the donors were grown on collagen gels for 7 days to evaluate survival and neurite outgrowth through RBPMS, cleaved-CASP3, and NEFL staining.

**Results:** Human iPSCs were successfully reprogrammed from donor keratocytes and differentiated into ROs that generated RGCs. Overexpression of NRN1 increased survival rates for non-glaucomatous RGCs ($p < 0.05$) compared to glaucomatous RGCs. In collagen gels, donor RGCs from both sources significantly reduced RGC apoptosis ($p < 0.05$) and increased neurite outgrowth after NRN1 treatment.

**Conclusions:** Our study confirmed that NRN1 enhances glaucomatous RGC survival. Thus, it supports the potential application of NRN1 as a therapeutic strategy for safeguarding RGCs against glaucomatous damage.

Keywords: Neuritin1, Glaucoma, Neuroprotection, Retinal Ganglion Cells

## POSTER 62

# ASYMMETRY IN THE ON AND OFF PHOTOPIC NEGATIVE RESPONSE OF THE FULL-FIELD ELECTRORETINOGRAM IN NON-HUMAN PRIMATE EXPERIMENTAL GLAUCOMA

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Evidence suggests differential ON/OFF pathway susceptibility in glaucoma, which offers pathophysiological insight to the mechanisms that result in irreversible death of retinal ganglion cells (RGCs). The photopic negative response (PhNR) of the full-field electroretinogram (ERG) detects RGC dysfunction with diagnostic utility that depends on stimuli, especially for flashes that separate light increment (ON) and decrement (OFF) responses. This study compares the ON and OFF PhNR to varied stimuli during light adaptation.

Full-field ERGs (BigShot, LKC) were recorded with contact lens electrodes sequentially in both eyes of N = 17 anesthetized rhesus macaques with unilateral experimental glaucoma (EG, ages 6-20 y). Eyes were dark adapted 20 mins before brief 4.5 ms and long 200 ms flashes, either red (560 cd/m2) on a steady blue background (30 cd/m2, R/B) or white on a white background (W/W), were presented every 2 mins for 20 mins of light adaptation. PhNR amplitudes were measured at 65 ms (ON) and 280 ms (OFF, for long flashes). Retinal nerve fiber layer thickness (RNFLT) was measured using OCT by a 12° circle scan (Spectralis). ANOVA compared PhNR amplitudes for varied conditions.

RNFLT loss ranged 5-71% in EG eyes (29.5 ± 20.3%, mean ± SD). PhNR amplitudes were reduced in EG eyes for brief R/B ($p < 0.0001$), long R/B (ON; $p < 0.0001$), and long W/W flashes (OFF; $p = 0.0002$). EG eyes produced significantly larger OFF PhNR amplitudes than control eyes for the long R/B flash ($p = 0.02$). PhNR amplitudes in EG eyes changed differently during light adaptation than in control eyes for brief W/W ($p = 0.03$), long R/B (ON; $p = 0.01$), and long W/W flashes (OFF; $p = 0.0004$).

EG may alter inner retinal circuitry that modulates ERG response amplitudes during light adaptation. The stimulus-dependent differences in the ON and OFF PhNR may suggest asymmetry in the pathway-specific contributions to the ERG in glaucoma with implications for diagnostic utility.

## POSTER 63

# EXTRACELLULAR VESICLE MEDITATED REMODELING OF THE TRABECULAR MESHWORK

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Active extracellular matrix (ECM) remodeling in the trabecular meshwork (TM) is critical to regulating aqueous humor outflow. Extracellular vesicles (EVs) are cellular nanoparticles that facilitate cell communication. In glaucoma, EVs have reduced ECM-associated proteins. We hypothesize that EVs regulate ECM structure and function and are necessary for normal TM physiology. To investigate our hypothesis, we used an EV biogenesis inhibitor, GW4869 and primary TM cells cultured from non-glaucomatous human donor eyes. We performed viability assays in three biological replicates over 24, 48, and 72 hours. To assess EV formation and TM protein expression, we cultured TM in EV-depleted media for 24, 48, and 72 hours with 15 or 30 μM GW4869 or vehicle (DMSO). EVs were purified from conditioned media through ultracentrifugation, then size and concentration was assessed via nanoparticle tracking analysis. Western blot was performed on cellular proteins, and antibodies for EGF-like repeats and discoidin I-like domain-3 (EDIL3), fibronectin, and α-smooth muscle actin (αSMA). Average cell viability surpassed 80% at all time points. Preliminary results demonstrated that 30 μM GW4869 reduced EV concentration by 36.3% at 24 hours and 79.6% at 72 hours compared to the untreated control (n = 1). EV size ranged from 131.6 to 232.6 nm. Expression of EDIL3 was increased at 24 hours with 30 μM GW4869 and 48 and 72 hours at both concentrations, with a maximal increase of 3.2 0.27-fold in the 72 hour 30 μM group (n = 3). Fibronectin and αSMA expression were not significantly impacted. Our results demonstrate that GW4869 can be used with minimal cytotoxicity, and that attenuating EV production leads to modifications in protein expression. In glaucoma, ECM dysfunction contributes to TM stiffening, preventing effective intraocular pressure control. Therefore, illuminating the functions of EVs in TM physiology is an essential step in understanding glaucoma pathogenesis.

## POSTER 64

# OVARIECTOMY IN YOUNG ANIMALS LEADS TO INCREASED INTRAOCULAR PRESSURE (IOP) BY MIDDLE-AGE

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Evidence suggests menopause modulates intraocular pressure (IOP) (Panchami et al., 2013; Qureshi, 1996), the only modifiable risk factor for glaucoma. Past work suggests that the early onset of menopause increases the risk of developing glaucoma (Fotesko et al., 2022; Vajaranant & Pasquale, 2012), while our recent work found an association between menopause and glaucoma diagnosis onset in women (Hogan et al., 2024). To understand the role of age and menopause on IOP, we investigated how early-onset menopause affects IOP as compared to aging alone.

Female Brown Norway rats (3 months of age) underwent surgically induced menopause (ovariectomy [OVX]; n = 5) or sham surgery (Sham; n = 5). Using rebound tonometry (Tonometer, Icare), we took weekly morning IOP measurements on both eyes (McDowell et al. 2022) over two months five weeks post-surgery (4 months of age), followed by a final measurement at middle-age (10 months); the animals were anesthetized with isoflurane. We then compared the average IOP at 4- and 10-months using a two-way ANOVA with a Bonferroni post hoc test (significance level $p < 0.05$). Data are presented as mean ± standard deviation.

We observed that the IOP of OVX animals at 10 months were significantly higher (19.4 ± 1.4 mmHg) compared to both the IOP of OVX (14 ± 1.2 mmHg) and Sham (15.2 ± 2.5) animals at 4 months ($p = 0.002$ and $p = 0.017$, respectively). OVX animals' IOPs trended towards higher values when compared to Sham animals at 10 months (19.4 ± 1.4 mmHg vs. 17.2 ± 2.2 mmHg; $p = 0.5$).

These results suggest that aging can increase IOP, and OVX at a young age may lead to higher IOP. Future work will increase the sample size, compare these results to older animals (20 months old), and explore the mechanisms potentially driving IOP increase including trabecular meshwork stiffness and outflow resistance.

## POSTER 65

# DIGITAL LIGHT PROCESSING 3D BIOPRINTING OF A SCALED UP GELMA-BASED LAMINA CRIBROSA MODEL FOR CELL CULTURE

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The lamina cribrosa (LC) is a key site of retinal ganglion cell (RGC) axon damage in glaucoma. There is currently a lack of a readily-available, structurally-accurate in vitro 3D cell culture model of the human LC. Our work is aimed at fabricating a 3D cell culture model of the LC using Digital Light Processing (DLP) 3D Bioprinting, offering a fast and scalable method of modelling the LC cells in vitro. High-resolution, histomorphometric 3D reconstructions of the human LC were used to create a biofidelic stereolithography computer model at 1.5 x 1.5 x 6 µm resolution. A BIONOVA X DLP 3D Printer was used to manufacture 3D culture scaffolds made from either Porcine GelMA (12%) or a mixture of Fish GelMA (12%) + Collagen (0.5%), scaled up 3.6x in size. The 3D-printed constructs had a similar appearance to the 3D computer model on gross examination and under light microscopy when the 3.6x size scaling was taken into account. The Porcine GelMA constructs showed an average compressive modulus of 5.49 ± 0.94 kPa, while the Fish GelMA + Collagen constructs showed an average compressive modulus of 20.77 ± 2.3 kPa, comparable to that of the normal LC. Rabbit conjunctival fibroblast cells were cultured on the 3D printed scaffolds, which resulted in significant differences in cell viability noted on live/dead staining between the two animals (82.3% live versus 15.2% live). DAPI and Phalloidin staining was used to assess cell adhesion, revealing cell nuclei to be located predominantly on the upper planes, with the cytoskeleton extending towards the interior of the scaffold. A key limitation of our model is that it is scaled-up in size compared to the in vivo LC. Future work exploring bioink formulations alongside advances in 3D bioprinting resolution will be key to overcoming this limitation.

## POSTER 66

# OCT BIOMARKERS AND CLINICAL SEVERITY IN JAMAICAN GLAUCOMA PATIENTS: A WINDOW INTO MISSED DETECTION

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**Introduction/Purpose:** Glaucoma among African and African Caribbean populations is distinct, with early age of onset, severe presentation, and poor outcomes being more common than in other ancestral groups. There remains limited population-specific data from the Caribbean, particularly Jamaica. This study presents the first clinical and imaging-based characterization of glaucoma among adult patients of Kingston Public Hospital (KPH), home to Jamaica's largest public Ophthalmology Clinic.

**Methods:** This retrospective clinical study included evaluation of patient charts (N = 324 paper records with complete visit histories), visual field (VF) test results, and optical coherence tomography (OCT) images and data. Disease staging followed the Global Glaucoma Staging System; diagnostic criteria followed the American Academy of Ophthalmology Preferred Practice Guidelines. OCT parameters across severity levels, adjusting for age, were evaluated with analysis of covariance; analyses were conducted in R (v4.4.1) with a significance threshold of $p < 0.05$ (two-sided).

**Results:** Average patient age was 58.3 ± 2.3 years, 66.6% were female, and 75% had at least one VF test. Early glaucoma (18.2%) was much less common than advanced glaucoma (51%). Patients with advanced glaucoma had significantly lower averages for several OCT parameters (RNFL thickness, rim area, cup-to-disc ratio, cup volume; $p < 0.05$) than those with early and moderate glaucoma. Ocular history data indicated most patients had prior cataract diagnosis (74.4%), many had prior eye trauma (26.5%), and reported a family history of glaucoma (30.9%).

**Conclusion:** Glaucoma severity was associated with distinct OCT changes, indicating progressive structural damage that remained significant even after accounting for age-related variation. This work offers insights into glaucoma presentation and severity in the Jamaican population accessing care at the largest public Ophthalmology Clinic in Jamaica. The high prevalence of moderate to advanced glaucoma in this population underscores gaps in early glaucoma detection and can serve to inform glaucoma diagnosis and management, while motivating early intervention strategies.

## POSTER 67

# NOGGIN PRESERVES RETINAL INTEGRITY IN A MURINE MODEL OF OCULAR HYPERTENSION

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Ocular hypertension is a condition defined by increased intraocular pressure (IOP). Over time, this can lead to the death of the retinal ganglion cells (RG) and thinning of the optic nerve, which are the leading contributors to primary open-angle glaucoma and irreversible blindness. Noggin is a BMP antagonist. It has been shown to prevent reactive gliosis and inflammation, which are important contributors to the progression of glaucoma. In this study, ocular hypertension was induced in mice by blocking the flow of aqueous humor through the injection of microbeads into the anterior chamber of the eye. One week after the initial microbead injection, 1 uL of noggin protein was injected intravitreally to evaluate its effect on two different parameters: the number of retinal ganglion cells remaining and the thickness of the nerve fiber layer. These parameters were recorded by using immunofluorescence and H&E staining. These findings highlight a potential therapeutic treatment for ocular hypertension management and glaucoma prevention.

## POSTER 68

# DIVERGENT ROLES OF HEALTHY AND GLAUCOMATOUS TRABECULAR MESHWORK-DERIVED EXTRACELLULAR VESICLES IN REGULATING EXTRACELLULAR MATRIX HOMEOSTASIS AND FIBROTIC SIGNALING

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Dysfunction of the trabecular meshwork (TM), marked by dysregulation of extracellular matrix (ECM), cytoskeleton and transmembrane receptors, contributes to elevated intraocular pressure (IOP), a risk factor in glaucoma. Small extracellular vesicles (sEVs), through their diverse molecular cargo, are emerging as critical modulators in glaucoma pathogenesis and therapy. We hypothesize that TM-derived sEVs regulate homeostasis of ECM components, cytoskeletal structure, and transmembrane receptors in outflow pathway tissues. sEVs were isolated from conditioned media of confluent non-glaucomatous (NTM) and glaucomatous (GTM) TM cells cultured in 1% EV-free serum, using iodixanol-cushioned ultracentrifugation. sEVs were characterized using Western blotting and nanoparticle tracking analysis (for size and concentration). Cellular uptake of fluorescently labeled sEVs was confirmed. Confluent NTM cells were treated for 7 days with dexamethasone (DEX; 500 nM) or vehicle (EtOH), with/without NTM-derived sEVs (2.08108). Separately, NTM cells were treated with GTM-derived sEVs (2.08108). Western blotting was used to assess expression of fibronectin (Fn), myocilin (MYOC), collagen alpha-1(IV) (COL4A1), alpha smooth muscle actin (αSMA), and integrins (β1 & β3). DEX treatment significantly upregulated Fn (2.94×, $p < 0.001$), MYOC (1.57×, $p < 0.05$), COL4A1 (1.59×, $p < 0.05$), αSMA (1.55×, $p < 0.05$), integrin β1 (1.46×, $p < 0.05$) and integrin β3 (1.73×, $p < 0.05$), compared to vehicle controls. Co-treatment with NTM-derived sEVs mitigated these effects, maintaining protein levels similar to controls: Fn (1.26×, $p = 0.69$), MYOC (1.17×, $p = 0.45$), COL4A1 (1.09×, $p = 0.38$), αSMA (1.09×, $p = 0.74$), integrin β1 (0.95×, $p = 0.51$) and integrin β3 (1.25×, $p = 0.35$). In contrast, GTM-derived sEVs induced a non-significant increase of Fn [1.39×, $p = 0.09$), MYOC (1.40×, $p = 0.14$), integrin β1 (1.21×, $p = 0.32$) and integrin β3 (1.24×, $p = 0.25$). These findings suggest that NTM-derived sEVs counteract DEX-induced fibrotic changes, whereas GTM-derived sEVs elicit a mild glaucomatous phenotype. This underscores the distinct functional roles of EVs from healthy and glaucomatous TM cells and supports their regulatory influence on ECM, cytoskeletal, and transmembrane receptor homeostasis.

## POSTER 69

# SREBP2-DEPENDENT TRANSCRIPTIONAL CONTROL OF LIPID METABOLISM IN TRABECULAR MESHWORK AND INTRAOCULAR PRESSURE REGULATION

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Recently we provided the evidence for mechanosensing and mechanotransduction in trabecular meshwork (TM) and intraocular pressure (IOP) regulation by the activation of lipogenic transcription factor sterol regulatory element binding proteins (SREBPs). Among the three different SREBPs, this study investigated SREBP2, which controls cholesterol synthesis pathway. Histochemical analysis of the human TM outflow pathway indicated increased nuclear localization of SREBP2 suggesting its activation in the ocular hypertensive subjects compared to normal. Injection of adenovirus constitutively expressing active or nuclear SREBP2 (AdN-SREBP2) in C57BL6 mice eye, resulted in a significant increase in intraocular pressure (IOP). To better understand the role of SREBP2 in the control of metabolism and provide a rationale for potential altered metabolic flux in TM leading to ocular hypertension, we performed an mRNA sequencing in human TM (HTM) cells constitutively expressing N-SREBP2. We found that among the significantly induced transcripts were glutamate-ammonia ligase/Glutamine synthetase (GLUL) and Glutaminase 2 (GLS2) - central to nitrogen metabolism and glutamine/a-ketoglutarate for fueling the TCA cycle, glutamate dehydrogenase 1 and 2 (GLUD1 &2), 3-hydroxy-3-methylglutaryl-CoA reductase (HMGCR) and fatty acid synthase (FASN) - rate limiting enzymes involved in mevalonate/cholesterol and fatty acid biosynthetic pathways, and ATP Citrate Lyase (ACLY), a critical metabolic enzyme that bridges carbohydrate metabolism and lipogenesis, energy homeostasis, and epigenetic regulation. Interestingly, SREBP2 activation upregulated integrin subunits (ITGA2B, ITGB1BP2), Rab-GTPases interacting proteins (RAB11FIP4 and 5) and chloride channel CLIC3 associated integrin a5b1 recycling cascade. Immunofluorescence localization and total internal reflection (TIRF) microscopy identified significant induction and presence of activated integrin b1 on TM membrane. This reveals that SREBP2 activation in the TM drives a metabolic shift toward cholesterol synthesis, glutamine metabolism, and lipogenesis, simultaneously upregulating integrin-mediated mechanotransduction pathways. These changes likely contribute to TM stiffening, ECM remodeling, and elevated IOP in glaucoma.

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## POSTER 70

# CIRCADIAN MODULATION OF AQUEOUS NOREPINEPHRINE LEVELS DRIVES OUTFLOW FACILITY RHYTHM IN RATS

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Intraocular pressure (IOP) in rats follows a circadian rhythm that reflects diurnal changes in aqueous humor dynamics. This study investigates anatomical and physiological mechanisms underlying the IOP rhythm. Experiments were performed on male, retired breeder Brown Norway rats entrained to a 12hr-light:12hr-dark cycle. Eyes of one cohort (n = 2) were enucleated and stained for DAPI, anti-tyrosine hydroxylase, and CD31 to fluorescently label ocular tissues, sympathetic nerve fibers, and Schlemm's canal region, respectively. IOP, outflow facility (C), and aqueous norepinephrine concentration [NE] were measured in another cohort (n = 7) during subjective day (8-11 AM) and night (8-11PM). [NE] was quantified via aqueous paracentesis using enzyme-linked immunosorbent assay (Eagle Biosciences EA633/96) and compared across four conditions (daytime in light, daytime in dark, nighttime, nighttime 1-hr after instillation of tetrodotoxin) using one-way repeated-measures ANOVA (post hoc: paired t-test). C was measured one week later via gravity-driven constant-pressure perfusion with animals in darkness under isoflurane anesthesia and redlight illumination. Immunohistochemical labeling indicated sympathetic innervation of ciliary body, iris, and aqueous drainage vessels, but not Schlemm's canal region. Nighttime IOP (30 ± 4 mmHg) was significantly higher than daytime IOP (15 ± 4 mmHg, $p < 0.001$) and was reduced to 16 ± 3 mmHg by tetrodotoxin ($p < 0.001$). Daytime [NE] in light (0.42 ± 0.1 ng/mL) and darkness were not different (0.54 ± 0.16 ng/mL, $p = 0.15$), but both were significantly lower than nighttime [NE] (1.97 ± 0.65 ng/mL, $p < 0.001$). Tetrodotoxin significantly lowered nighttime [NE] (0.47 ± 0.23 ng/mL, $p < 0.001$) to levels that were indistinguishable from daytime [NE] ($p = 0.59$). C increased significantly from day (12.6 ± 1.4 nl/min/mmHg) to night (31.7 ± 13.5nl/min/mmHg) and correlated with [NE] ($r^2 = 0.45$). Aqueous [NE] follows a circadian rhythm, which correlates in magnitude with C. The circadian rhythm in IOP and C is thereby mediated, in part or whole, by aqueous [NE] carried from ciliary body processes to the proximal trabecular outflow pathway and perhaps to direct sympathetic [NE] input to the distal outflow pathway.

## POSTER 71

## CYP1B1 AND POSTN IN TRABECULAR MESHWORK DEVELOPMENT

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Genetic analyses have identified Cyp1b1 as a major gene associated with early-onset glaucoma, with mutations present in approximately 20% of PCG cases. Mouse models with Cyp1b1 deletion recapitulate key developmental abnormalities in TM and SC observed in human PCG. Notably, Cyp1b1 deletion in mice disrupts the expression of Postn (Periostin), a matricellular protein that signals through integrins and has been implicated in extracellular matrix remodeling. However, the precise roles of Cyp1b1 and Postn in TM development and their mechanistic contributions to glaucoma pathogenesis remain poorly understood. Our previously generated single-cell RNA sequencing-based developmental atlas of the mouse anterior segment revealed a dynamic relationship between Cyp1b1 and Postn expression during TM development. Preliminary data from our lab in adult mice with combined deletion of Cyp1b1 and Postn ($Cyp1b^{0/0}$; $Postn^{0/0}$) mice show significant TM thinning. We previously identified three distinct TM subtypes (TM1–TM3), marked by Myoc, Crym, and αSMA, respectively. Our current studies seek to understand the loss of specific TM subtype(s) in $Cyp1b^{0/0}$; $Postn^{0/0}$ with implications in understanding their individual role in TM function, IOP elevation, and congenital glaucoma.

## POSTER 72

# ROLE OF KLF4 IN SENESCENCE DYNAMICS IN GLAUCOMATOUS TRABECULAR MESHWORK CELLS

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Glaucoma, a leading cause of irreversible blindness, is characterized by trabecular meshwork (TM) dysfunction, a tissue integral for intraocular pressure regulation. Although cellular senescence in the TM has been identified as a major cause of glaucomatous disease, the molecular regulators directing this process are still not fully understood. This study explores the involvement of the transcription factor Krüppel-like factor 4 (KLF4) in the modulation of senescence-associated and proliferative signaling in human TM cells under stress conditions.

Human TM cells isolated from donor corneoscleral tissue were characterized by standard markers following dexamethasone exposure. To induce a pathophysiologically relevant stress response, cells were exposed to transforming growth factor-beta (TGF-β). The expression profiles of KLF4, senescence markers (p16, p27, and p53), and cell proliferation regulators (cyclin D1 and PCNA) were evaluated using quantitative RT-PCR and immunofluorescence.

Cells exposed to TGF-β displayed morphological and molecular characteristics suggestive of a senescence-like phenotype. Senescence and proliferation-associated factors' transcript and protein levels were altered with changes in KLF4 expression, suggesting a possible link between KLF4 activity and the cellular fate of TM cells under stress.

These findings suggested KLF4 as a crucial transcriptional regulator in the dynamic equilibrium between TM cell growth and senescence, highlighting its potential as a therapeutic target for preserving TM function and homeostasis in glaucoma.

## POSTER 73

# YEAST AS A MODEL EUKARYOTIC ORGANISM FOR MYOCILIN-INDUCED GLAUCOMA CYTOTOXICITY

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Glaucoma is the second leading cause of irreversible blindness affecting over 80 million people around the world, with mutant myocilin-associated glaucoma accounting for 2-4% of POAG cases, and 10-30% of JOAG cases. Myocilin is a protein expressed in the trabecular meshwork (TM) in the eye at a relatively high level. Mutant myocilin undergoes misfolding and aggregation within TM cells, causing endoplasmic reticulum (ER) stress and cell death. As TM cells are a component of the trabecular meshwork responsible for maintaining intraocular pressure, TM cell death hastens TM dysregulation and neurodegeneration within the optic nerve. Little is known about the underlying cause of cytotoxicity from mutant myocilin in TM cells. In this project, the simple eukaryote Saccharomyces cerevisiae, commonly known as Baker's yeast, was utilized to generate a model of mutant myocilin cytotoxicity, paralleling studies of other misfolding diseases. Transforming yeast with both wild-type myocilin and pathogenic myocilin mutants yields different levels of cytotoxicity. We screened a yeast knockout library to identify potential genes associated with cytotoxicity caused by myocilin. We found that genes within autophagy, ubiquinome, and neurodegenerative disease pathways affect myocilin toxicity. In parallel, screening an FDA-approved compound library led to the identification of small compounds that rescue cytotoxicity caused by myocilin. Altogether, our work developing a yeast model of myocilin toxicity has led to the identification of new genetic factors that sensitize cells to myocilin toxicity and small molecules with the potential to be developed into therapeutics for myocilin-associated glaucoma. Our current work is focused on translating our findings in yeast to TM cells and animal models.

## POSTER 74

# CELLULAR TRAFFICKING OF GLAUCOMA-ASSOCIATED MYOCILIN VARIANTS OF UNCERTAIN SIGNIFICANCE

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Missense mutations in MYOC are the strongest genetic link to primary open-angle glaucoma. Bona fide glaucoma-causing mutations, housed in the C-terminal olfactomedin (OLF) domain, are familial, rare, and cause early-onset vision loss. Disease mutations introduce a toxic gain of function within trabecular meshwork (TM) cells, hallmarked by non-secretion with misfolding and aggregation intracellularly in the endoplasmic reticulum (ER). Chronic ER stress eventually leads to TM cell death, hastening disease progression. Here we compared A427T, a missense variant with marginal stability and Q368X, a premature stop highly prevalent in the population, to disease missense mutant Y437H. Immortalized human TM-1 cells were stably transduced with wild-type (WT) MYOC and MYOC variants Y437H, A427T, and Q368X. While WT MYOC was secreted, the three variants exhibited little if any secretion and instead exhibited different extents of soluble and insoluble intracellular accumulation. Inhibition of autophagy with BafA1 increased insoluble WT MYOC levels while proteasomal inhibition by MG132 increased colocalization with lysosomal marker atg5, suggesting that WT MYOC degradation involves autophagy to a degree not previously appreciated. By comparison, TM cells attempt to clear different MYOC variants by the additional use of the proteasome. However, neither pathway is sufficient to completely clear intracellular MYOC variants to maintain proteostasis. Results support the promotion of autophagic clearance and proteasomal degradation presents a potential therapeutic and the use of genetic screening to assess glaucoma risk in the clinic.

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## POSTER 75

# MODULATING UNDESIRED TM CELL MISLOCALIZATION WITHIN 3D TM/SC CELLCO-CULTURE HYDROGELS BY USING PERMEABLE SUBSTRATES

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Dynamic interactions between the trabecular meshwork (TM) and Schlemm's canal (SC) inner wall govern aqueous humor outflow homeostasis. We recently designed a 3D TM/SC cell co-culture hydrogel model that recreates critical aspects of the native tissue interface: (i) human TM cells are encapsulated within a tissue-like ECM hydrogel adhered to a solid substrate, (ii) basement membrane proteins are surface-coated, (iii) human SC cells are seeded atop. Only negligeable SC cell migration into the underlying hydrogel was noted over time (~5%; N = 3 cell strains). However, a key challenge was TM cell mislocalization due to nutrient gradients near the hydrogel surface created by the impermeable substrate. We found up to 50% encapsulated TM cells gradually migrated/concentrated near the hydrogel surface immediately below the SC cell layer, making it difficult to differentiate the two cell types. Here, we hypothesize that using permeable 0.4 µm Transwell culture inserts reduces TM cell mislocalization due to media flow through the co-culture hydrogels. We observed decreased TM cell density at the hydrogel surface over time (~10-25%; N = 3 cell strains) with permeable inserts compared to impermeablized controls ($p < 0.05$), independent of the presence (= co-culture) or absence (= acellular hydrogels) of SC cells. The TM cell number in the hydrogel center showed the inverse relationship ($p < 0.05$) and no differences were noted between groups in the hydrogel substrate layer. To further improve accurate cell tracking, we labeled SC cells with BrdU and TM cells with Click-iT EdU prior to fabricating the TM/SC cell co-culture hydrogels. We showed ~85-95% differential labeling efficiency to distinguish SC cells from migrated TM cells in 3D using this technique. These data suggest that undesired TM cell mislocalization within the TM/SC cell co-culture hydrogels can be overcome by using permeable Transwell inserts, setting the stage for more accurate investigations of cellular crosstalk and response to different stimuli.

## POSTER 76

# ACTIVATION OF THE WNT SIGNALING PATHWAY INHIBITS STEROID-INDUCED OCULAR HYPERTENSION WITHOUT COMPROMISING ITS ANTI-INFLAMMATORY FUNCTIONS IN THE MOUSE MODEL

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Steroids are widely used as anti-inflammatory medications. Long-term steroid use causes side effects such as elevated intraocular pressure (IOP) in about 40% of the population, a condition called steroid-induced ocular hypertension (OHT). Therefore, it is crucial to inhibit steroid-induced OHT without compromising their therapeutic use. Our published studies suggested that inhibition of the canonical Wnt signaling pathway plays a role in steroid-induced OHT, and we hypothesize that activation of canonical Wnt signaling prevents the side effects of steroids without compromising their therapeutic effects. To study this, we injected TCF-GFP mice (a Wnt signaling reporter line) and β-catenin conditional knockout mice with or without a vehicle or dexamethasone (DEX) periocularly weekly. Some DEX-treated mice also received CHIR (a canonical Wnt signaling activator) eye drops. The TCF-GFP mice receiving DEX showed OHT compared to the vehicle control mice, whereas those receiving CHIR and DEX showed a significant decrease in IOP compared to the DEX alone mice. Immunostaining showed an increase in Wnt signaling-driven GFP expression in CHIR-treated eyes. Further, β-catenin conditional knockout mice showed persistent OHT even when they were treated with CHIR, suggesting that canonical Wnt signaling activation is required to alleviate DEX-induced OHT. We also treated wildtype C57BL/6J mice that developed endotoxin-induced uveitis (EIU) or experimental autoimmune uveitis (EAU) with or without DEX and/or CHIR. Their ocular inflammation was evaluated using aqueous humor protein concentrations or the EAU scoring system based on OCT and fundus image changes, respectively. We found that DEX and DEX+CHIR had similar effects on the suppression of uveitis. Overall, our data suggest that canonical Wnt signaling activation inhibits DEX-induced OHT without compromising its anti-inflammatory activity in mouse eyes.

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## POSTER 77

# DEVELOPMENT AND CHARACTERIZATION OF A THREE-DIMENSIONAL HUMAN LAMINA CRIBROSA AND OPTIC NERVE HEAD ASTROCYTE CO-CULTURE SYSTEM

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In chronic glaucoma, the human optic nerve head (hONH) undergoes fibrosis, characterized by extracellular matrix (ECM) deposition and stiffening. The major pro-fibrotic cells in this region are lamina cribrosa (LC) cells and ONH astrocytes (ONHAs). Critical gaps in our understanding of glaucoma pathobiology include determining which of these two cell types are the primary sensors of glaucomatous stimuli, and their individual contributions to tissue fibrosis. A key limitation of existing in vitro hONH cell models is the lack of an adequate ECM scaffold and an over-reliance on 2D culture systems that poorly recapitulate essential features of the hONH. Here, we developed a novel co-culture model of primary LC-ONHA cells using a physiologically relevant 3D ECM hydrogel system. To determine the contribution of each cell type to fibrosis, we encapsulated both LC and ONHAs at different proportions (LC:ONHA ratios were as follows in %: 100/0, 91/9, 9/91, 0/100). LC+ONHA cells were encapsulated within hydrogels comprised of 3.1 mg/ml collagen I, 1 mg/mL hyaluronic acid, and 0.06% photoinitiator LAP, and crosslinked with blue light (10s exposure, 320 mW/cm2) to achieve a target stiffness of ~0.3 kPa as measured by rheology. LC and ONHA cells within the hydrogel exhibited morphologies distinct from the cells in 2D culture; LC cells were primarily polygonal-bodied with multiple thick processes, whereas ONHAs had smaller stellate morphologies with a spheroid core and thin spiny processes. We further treated LC+ONHA co-cultures with the known glaucomatous stressor TGFβ2 (5 ng/mL) for 48 hours. Both LC and ONHA-containing TGFb2-treated hydrogels developed higher expression of total fibronectin and fibronectin-EDA than untreated controls. Taken together, our preliminary data supports usage of our 3D ECM hydrogel system as a viable tool to investigate the mechanisms of hONH cellular crosstalk and fibrosis in glaucoma.

## POSTER 78

# ASSESSING MOLECULAR AND FUNCTIONAL CONSEQUENCES OF THE GLAUCOMA-ASSOCIATED APBB2 SNP USING HUMAN INDUCED PLURIPOTENT STEM CELL MODELS

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Glaucoma has been found to occur more frequently and with higher severity in certain populations, yet research into the biological basis for these differences in susceptibility are lacking. A recent GWAS study identified a SNP in the APBB2 gene that was linked to increased risk for glaucoma uniquely within specific populations, and the discovery of this SNP provides an exciting opportunity to study why those populations are at increased risk for glaucoma. To examine how a SNP at the APBB2 locus may confer increased risk for glaucoma, we leveraged CRISPR/Cas9 gene editing approaches to generate isogenic human induced pluripotent stem cell (iPSC) lines with paired control and ABPP2 variant genetic backgrounds. Each of these cell lines were capable of robust differentiation into retinal organoids, which were then used for the purification and maturation of RGCs for downstream analyses. Upon differentiation and maturation of RGCs, results indicated that those with the APBB2 SNP exhibited significantly decreased neurite complexity and increased neuronal excitability, suggestive of disease-related features. Ongoing experiments are focusing upon assessing transcriptional changes as a consequence of this APBB2 variant, as well as how this variant may alter amyloid processing. These studies represent the first known attempt to develop iPSC-based models for the study of increased risk for glaucoma due to the APBB2 genetic variant, with these results likely to elucidate important cellular and molecular aspects that uniquely lead to this increased risk for glaucoma.

## POSTER 79

# EVALUATION OF IRIS WATER CONTENT AND HISTOMORPHOLOGY IN PRIMARY ANGLE-CLOSURE GLAUCOMA

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Iris plays a key role in primary angle-closure glaucoma (PACG). The structure and mechanics of the iris, as well as their connection to PACG, are not well understood. Our objective was to measure iris water content and histomorphology, which are parameters affecting iris biomechanical properties.

We used fresh eyes from 6-month-old NZW rabbits. We measured the limbus and pupil diameters before dissection, and then carefully isolated and bisected the iris, using one half for measuring water content and the other half for histological analysis. We measured the water content as $(m_{wet} - m_{dry})/m_{wet}$ where $m_{wet}$ is the wet mass (±0.1 mg) and $m_{dry}$ is the dry mass (oven-dried at c. 63°C for four days). For histomorphology, we used Trichrome staining of radial cross-section slides. The average limbus/pupil diameters, % dilation (the ratio of pupil to limbus diameter), water content, and sphincter muscle area were calculated and compared between male and female groups (unpaired t-test, $\alpha = 0.05$).

The following measurements were obtained (mean ± standard deviation): limbus diameter was 15.1 ± 0.9 mm in males (n = 16) and 14.8 ± 0.7 mm in females (n = 17); pupil diameter was 8.4 ± 1.4 mm (male) and 8.0 ± 0.8 mm (female), respectively, resulting in postmortem dilation: 55.4% ± 9.0% (male) vs. 53.5% ± 4.6% (female). In addition, water content was high in both groups: 88.2% ± 3.3% (n = 19; male) vs. 86.6% ± 2.2% (n = 20; female). Lastly, sphincter muscle areas were similar: $5.3\times10^4 \pm 1.8\times10^4$ (n = 8; male) vs. $5.8\times10^4 \pm 2.4\times10^4$ (n = 9; female). Our analysis did not detect any sex differences ($p > 0.1$).

Our study provides the first account of benchmark values for iris water content, combined with its muscular morphology, which can be used in the evaluation of iris biomechanical properties during tensile biomechanical testing and finite element analyses. Finally, although the rabbit model is advantageous due to its round pupil, we aim to expand this study to more clinically-relevant samples using cadaveric human eyes to investigate the role of iris biomechanics in PACG.

## POSTER 80

# TRANSCELLULAR DEGRADATION OF AXONAL MITOCHONDRIA AND AMYLOID BETA BY HYPERPHAGOCYTIC ASTROCYTES

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The Marsh-Armstrong laboratory previously discovered a process by which retinal ganglion cell (RGC) axons shed axonal mitochondria in the optic nerve head of mice, and recently showed, using an optic nerve live imaging-based assay in Xenopus laevis tadpoles, that the process is increased by expression of glaucoma-associated mutant Optineurin. Here we report that a similar transcellular clearance from axons occurs in Xenopus laevis tadpoles for the Alzheimer's-associated amyloid beta (Ab). Intravitreal injection of fluorescently labeled Ab results in the accumulation of Ab outside of axons. Interestingly, the externalized Ab is not uniform but rather shows discrete foci. These foci are within discrete astrocytes, here referred to as hyperphagocytic astrocytes, or in myeloid cells. Within the hyperphagocytic astrocytes, the externalized Ab largely localizes to lysosome-related organelles that are labeled by astrocyte-expressed CD63-mCherry. The degree of co-localization at different times after intravitreal injection will be presented, along with preliminary studies that assess externalized Ab with an antibody after RGC-specific conditional expression of Alzheimer's-associated amyloid precursor protein. The goal of these studies is to understand how organelles and protein aggregates are cleared from retinal ganglion cell axons with the ultimate goal of identifying therapeutic interventions that are applicable to various neurodegenerative diseases, not just glaucoma.

## POSTER 81

# SINGLE-SCAN 3D MAPPING OF RETINAL NERVE FIBER LAYER PLEXUS USING FREQUENCY-DOMAIN FREQFORMER AND COMMERCIAL NIR-OCT

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Early and precise characterization of retinal nerve fiber layer (NFL) alterations is critical for glaucoma detection and monitoring. However, commercial near-infrared optical coherence tomography angiography (NIR-OCTA) systems often suffer from low signal-to-noise ratio (SNR) and limited resolution in deeper or fine vascular structures, including the NFL plexus and adjacent nerve fiber bundles. To address this challenge, we introduce Freqformer, a dual-branch Transformer-based neural network designed to enhance 3D retinal vascular architecture from a single commercial NIR-OCT scan. Freqformer integrates global spatial context through a modified Transformer encoder and selectively amplifies frequency components via a complex-valued frequency-domain module (CFDM), enabling superior reconstruction of capillary continuity and fine vasculature. Our method was validated on human NIR-OCTA volumes acquired with a commercial swept-source OCT system. Results show that Freqformer significantly improves SNR, structural sharpness, and capillary segment connectivity compared to existing CNN and Transformer baselines. Critically, Freqformer enables clear 3D visualization and layer-specific quantification of the NFL plexus, a region closely associated with early glaucomatous damage. Enhanced reconstructions reveal finer capillary branches and nerve fiber bundle patterns that are otherwise difficult to resolve. Quantitative metrics - including capillary density, segment length, and tortuosity - demonstrate high consistency with merged multi-scan references, while requiring only a single scan. Our findings support Freqformer's clinical utility in routine NIR-OCTA imaging, enabling improved assessment of NFL vasculature and potentially nerve fiber architecture in glaucoma patients. The method's compatibility with commercial hardware and minimal computational overhead makes it highly applicable for future integration into diagnostic workflows.

## POSTER 82

# AMPLIFICATION OF HOMEOSTATIC PROSTAGLANDIN $D_2$ SIGNALING PROMOTES NEUROPROTECTION IN RETINAL NEURODEGENERATION MODELS

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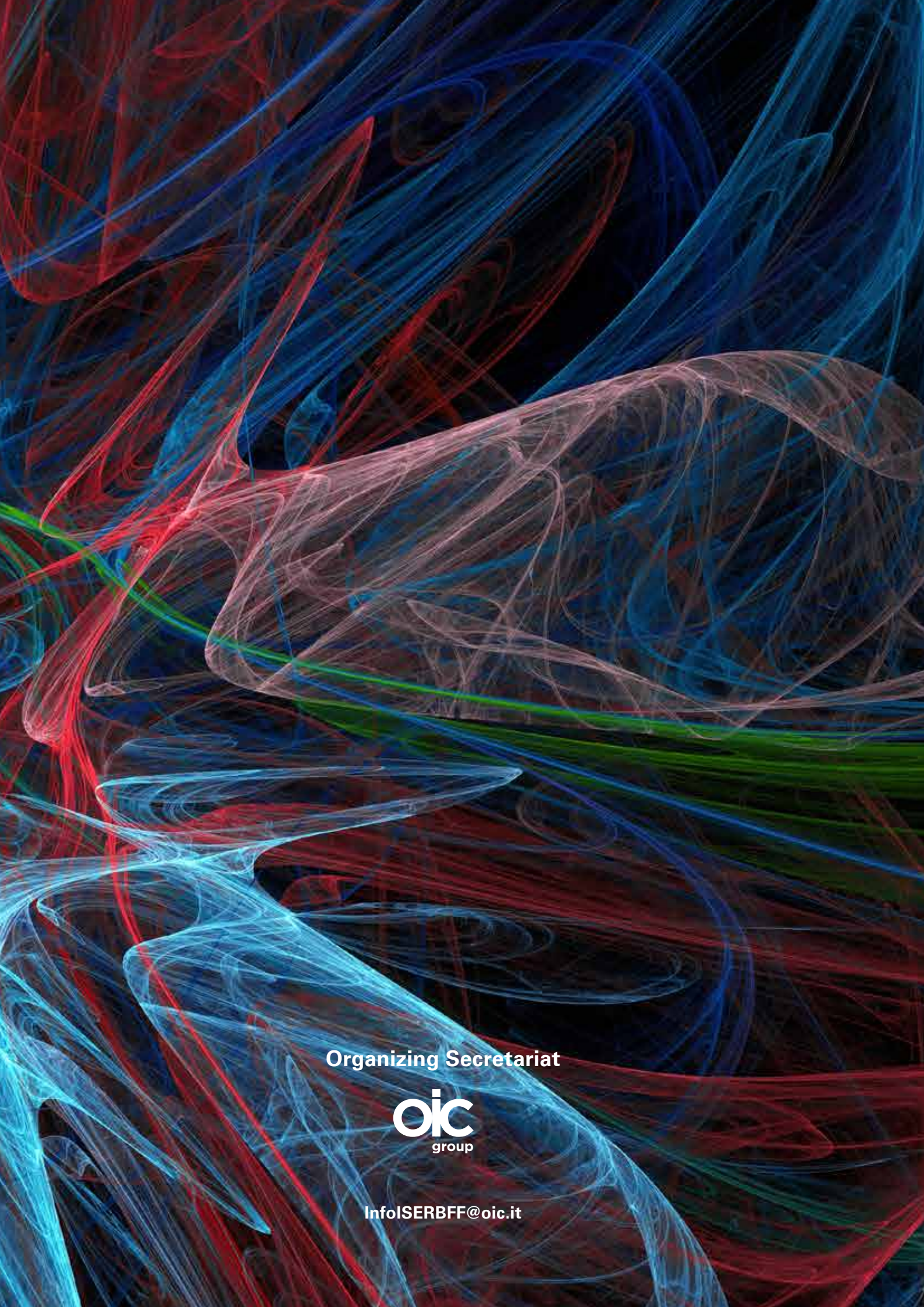
Despite the widespread clinical use of prostaglandin analogs such as PGE2 and PGF2α to lower intraocular pressure (IOP), the endogenous roles of prostaglandins in retinal neuroprotection remain poorly understood. We recently discovered Prostaglandin D2 (PGD2) as the most abundant constitutive prostaglandin in the healthy retina and optic nerve, with levels nearly 200-fold higher than other prostanoids. Bulk RNA sequencing of healthy retina and optic nerve from mouse and macaque confirmed expression of key components of PGD2 signaling axis, including biosynthetic enzymes and receptors. All subsequent functional studies were performed in the mouse retina. PGD2 signals through two G-protein-coupled receptors—DP1 and DP2. Using in situ hybridization, western blotting, and immunohistochemistry, we found that both receptors are expressed in retinal ganglion cells (RGCs) and the inner nuclear layer, with DP1 showing higher expression levels than DP2. In a silicone oil–induced mild ocular hypertension (OHT) model, PGD2 levels were reduced by ~50%, accompanied by downregulation of its biosynthetic enzyme lipocalin-type PGD synthase (L-PGDS) and both DP1 and DP2 receptor expression. In additional loss-of-function studies, topical treatment with the nonsteroidal anti-inflammatory drug (NSAID) bromfenac, which inhibits prostaglandin production, reduced retinal PGD2 by 90% and significantly exacerbated RGC degeneration, glial activation, and optic nerve axonal loss compared to untreated OHT controls. In contrast, gain-of-function studies involving intravitreal delivery of PGD2 methyl ester or selective DP1/DP2 receptor agonists conferred robust neuroprotection in a kainic acid–induced excitotoxicity model. Notably, DP1 agonist treatment resulted in the greatest RGC preservation, followed by DP2 agonist, suggesting effective receptor activation and a dose-dependent protective effect. These findings identify a previously unrecognized, homeostatic PGD2–DP1/DP2 signaling axis that plays a critical role in preventing RGC degeneration and support its therapeutic potential in glaucoma and optic neuropathies.



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