16th MIDWEST EYE RESEARCH SYMPOSIUM

Friday August 9, 2024

Abstracts

The organizing committee would like to give a special thanks to the University of Iowa Department of Ophthalmology and Visual Sciences and Research to Prevent Blindness for their financial and in-kind support of this meeting.

Schedule

8:15-8:45 Poster Set-up / Coffee
8:45-9 Opening Remarks

9-10 Platform Session I, Chaired by Elizabeth Berger, Ph.D.

9:00-9:15	Elizabeth Berger:	The Journey of Thymosin Beta 4: From Discovery to Innovations in Corneal Infection
9:15-9:30	Zachary Shepard:	Investigating the Presence of Ferritinophagy in Fuchs Endothelial Corneal Dystrophy
9:30-9:45	Virginia Mathu:	Effect of Unilateral Optic Nerve Crush and Microbead Injection on Optomotor Response Ratio in 5XFAD and wt C57BL/6J Mice
9:45-10:00	Karl Kador:	Using 3D bioprinting to direct retinal ganglion cell growth and create vasculature in engineered retinal constructs

10-11 Poster Session I/Coffee

11-12 Platform Session II, Chaired by Machelle Pardue, Ph.D.

11:00-11:15	Machelle Pardue:	Detection and Treatment of Early-stage Diabetic Retinopathy
11:15-11:30	Jordan Mayberry:	Elevated IOP facilitates T cell mediated RGC loss
11:30-11:45	Hu Huang:	Interferon Regulatory Factor 1 (IRF1) in Retinal Neurodegeneration
11:45-12:00	Gillian McLellan:	Neurofilament light chain as a biomarker of neurodegeneration in feline glaucoma

12-1 Lunch

1-2 Platform Session III, Chaired by Elliott Sohn, M.D

1:00-1:15	Elliott Sohn:	Integrated Systemic Inflammation and AI-Driven OCT Metrics in Neovascular Age-Related Macular Degeneration: Bridging Clinical Correlations and Pathophysiology
1:15-1:30	Kristin Davis:	Nystagmus recordings and correlation with underlying diagnosis in children
1:30-1:45	Apurva Dusane:	Rho-kinase inhibitor nanomedicine for Fuchs endothelial corneal dystrophy
1:45-2:00	Arlene V. Drack:	A candidate gene therapy vector restores cone function in a mouse model of Bardet-Biedl Syndrome Type 10

2-3 Poster Session II/Coffee

3-4 Keynote Address – Leonard Levin, M.D., Ph.D
"Axon Thriller - Dissecting the Plot of the Next Polywood Blockbuster"

4-4:30 Recognition of Outstanding Presentations

PLATFORM SESSION I

CHAIR: DR. ELIZABETH BERGER

9-10 PM

The Journey of Thymosin Beta 4: From Discovery to Innovations in Corneal Infection

Berger, Elisabeth

[1]WAYNE STATE UNIVERSITY

Purpose: This presentation aims to provide a historical perspective on the development of thymosin beta 4 (Tβ4) and highlight the current advancements in our laboratory for the treatment of corneal infections.
Methods: A targeted literature review was carried out regarding the discovery and early research of Tβ4. Current experimental approaches in our lab include an in vivo mouse model of P. aeruginosa-induced corneal infection accompanied by in vitro studies to evaluate the efficacy of Tβ4 as an adjunctive therapy to antibiotics. A variety of molecular and cellular techniques (RT-PCR, flow cytometry, Western blot) are employed to investigate the underlying mechanisms. These are paired with behavioral assessments to establish therapeutic potential.
Results: Tβ4 was initially identified for its role in actin regulation and wound healing. Recent studies in our lab have demonstrated the therapeutic potential of Tβ4 as an adjunct to antibiotics, showing significantly improved disease response following infection. In vivo results further show a role for Tβ4 in corneal wound healing, regulating effector cell function, and activating pro-resolving pathways critical to the restoration of corneal structure and function.
Conclusions: The historical significance of Tβ4 as a multifunctional peptide has paved the way for its current application as an adjunctive treatment for corneal infection. Our ongoing research highlights its potential as a therapeutic agent, offering promising results in enhancing corneal healing and resolving infection. Future studies will focus on exploring its efficacy against other pathogens and advancing Tβ4 into clinical trials.

Investigating the Presence of Ferritinophagy in Fuchs Endothelial Corneal Dystrophy

Shepard, Zachary[1,2]; Greiner, Mark[1,2]; Skeie, Jess[1,2]; Eggleston, Tim[1,2]; Shevalye, Hanna[2]

[1]CARVER COLLEGE OF MEDICINE, DEPARTMENT OF OPHTHALMOLOGY, UNIVERSITY OF IOWA; [2]IOWA LIONS EYE BANK

Purpose: Fuchs endothelial corneal dystrophy (FECD) is a progressive disease that causes premature death of corneal endothelial (CE) cells. Despite the global impact of FECD as the most common disorder of the corneal endothelium, relatively little is known about the mechanisms contributing to disease progression. Based on preliminary data demonstrating that iron-dependent lipid peroxidation mediates cell death in FECD, we hypothesized that FECD progression is mediated by alterations in ferritinophagy, a selective form of autophagy that contributes to ferritin degradation. This triggers labile iron overload, reactive oxygen species accumulation, lipid peroxidation, and cell death. The presence of ferritinophagy can be tracked through RNA and protein quantification of cellular markers NCOA4, LC3, and ferritin. We also hypothesized that ultraviolet-A (UV-A) light exposure, which has been demonstrated to contribute to both FECD progression and ferroptosis, participates in altered ferritinophagy.
Methods: We utilized control and FECD cells from both cultured immortal cell lines at the Iowa Lions Eye Bank. For human samples, we utilized patient derived DMEK peels (collected at time of FECD surgery) and healthy donor tissues. Cells were treated with various doses of UV-A and antimycin A (AMA) to induce oxidative damage. Protein and RNA markers of ferritinophagy were extracted and analyzed using PCR and microfluidics western blot to quantify NCOA4 and LC3 cellular levels. Additionally, treated cells were plated on coverslips, stained via immunohistochemistry, and analyzed using confocal microscopy to further determine cellular changes that may be associated with ferritinophagy in FECD.
Results: Immortalized cell lines affected with FECD demonstrated increased protein levels of the ferritinophagy markers NCOA4 and LC3 when compared to control cells, indicating that iron accumulation contributed to the cell death characteristic of FECD. This increase of NCOA4 protein levels in FECD was further validated in donor samples of diseased and healthy cells. UV-A exposure further increased the protein and mRNA levels of NCOA4 and LC3 present in FECD cell cultures, highlighting the effect of UV-A on ferritinophagy in FECD. Finally, on immunohistochemistry, ferritin and LC3 were noted to colocalize in a non-punctate pattern within the nuclei of cells with FECD.
Conclusions: Our results reinforce the role of ferritinophagy in FECD by demonstrating an increase of ferritinophagy markers NCOA4 and LC3 in FECD cells, both from immortalized cell lines and from donor samples. Additionally, this study provides a mechanism for the contribution of UV exposure to disease development, furthering the

current understanding of how FECD contributes to cell death and vision loss. Lastly, based on our immunohistochemistry studies, our results indicate a potential mechanism that cells with FECD enact to protect against nuclear oxidative damage from Fenton reactions.

Effect of Unilateral Optic Nerve Crush and Microbead Injection on Optomotor Response Ratio in 5XFAD and wt C57BL/6J Mice

Mathu, Virginia[1] Burkhalter, Rebecca[2]; Thao, Mason[2]; Kragerud, Ben[2]; Kiland, Julie A[2]

DEPARTMENTS OF [1]OPHTHALMOLOGY AND VISUAL SCIENCES; [2]SURGICAL SCIENCES, UNIVERSITY OF WISCONSIN-MADISON

Purpose: Optomotor response (OMR), a reflex used to assess visual function in animals, has not been well characterized in either the optic nerve crush (ONC) or the magnetic microbead (MMB) model of ocular hypertension, two common mouse models of optic neuropathy. Additionally, it has seldom been used to describe visual function in 5XFAD mice, a model of familial Alzheimer's disease (AD) due to β-amyloid accumulation. Here we combine the 5XFAD model of AD with ONC or MMB injection and measure their effects on visual function using OMR testing.

Methods: At 9 mths of age, OMR ratios were measured in 5XFAD (n=97; male n=56, female n=41) and wt C57BL/6J littermates (n=79; male n=43, female n=36) mice (qOMR system, Phenosys). Subsets of mice had undergone ONC (5XFAD n=26, WT n=22), sham surgery (5XFAD n=24, WT n=18), magnetic microbead injection (3 intracameral injections over 6 mths; 5XFAD n=12, WT n=15) or saline injection (5XFAD n=17, WT n=11), with all interventions initiated at 3mo. Mice were exposed to rotating visual stimuli of the following spatial frequencies: 0.2, 0.25, 0.3, 0.35, 0.4, 0.45, 0.5, and 0.6 cycles per degree (cpd). Mice were exposed to the stimuli 4 times (over two testing days) and optomotor response ratios averaged from these testing sessions for each eye.

Results: OMRs ratios at 0.2cpd were statistically significantly reduced in ONC eyes compared to naïve control eyes (5XFAD p±0.0001, wt p±0.0001) and compared to sham surgery controls (5XFAD p±0.0001, wt p±0.0001). Responses were also diminished in ONC eyes compared to contralateral eyes in both the 5XFAD and WT animals (p±0.0001, p±0.0001 respectively), with statistically significant differences persisting through 0.35 cpd. MMB injection also reduced OMR ratios but this difference was only statistically significant in MMB-injected wt eyes compared to wt naïve eyes at 0.2 cpd (p=0.0212). No statistically significant differences in OMR ratios were detected between naïve wt and naive 5XFAD mice, or between male and female mice in any group at 9 mo.

Conclusions: Quantitative OMR testing represents a viable non-invasive method to quantify unilateral reduction in visual function in awake 5XFAD and WT mice following ONC or MMB injection.

Using 3D bioprinting to direct retinal ganglion cell growth and create vasculature in engineered retinal constructs

Kador, Karl[1] Abukunna, Fatima[1]
[1]DEPARTMENT OF OPHTHALMOLOGY, UNIVERSITY OF MISSOURI-KANSAS CITY

Purpose: Self-forming retinal organoids (RO) have become a valuable tool in the study of retinal degenerative diseases as well as source for cell replacement therapies due to their ability to recreate all the cells of the neural retina in their correct cell lamina. However, these RO fail to create a properly organized retinal nerve fiber layer (NFL) or optic nerve (ON) due to the retinal ganglion cells (RGC) growing out from the RO, limiting their ability to be used as a model of glaucoma. In addition, these RO lack vasculature limiting their use as a model for diseases such as diabetic retinopathy. Interestingly, during development, the formation of the NFL, the ON and vasculature in the neural retina are linked in a common mechanism in which ON cells secrete factors which polarize the RGC growth before the ON head astrocytes (ONHA) migrate along the RGC axons while secreting VEGF to direct vascular cell migration. In this study, we use 3D bioprinting to recreate this mechanism in vitro.
Methods: RGCs were isolated from early postnatal Sprague Dawley rats (postnatal day 2-5) and purified through a two-step immunopanning protocol.
ONHA were isolated from postnatal day 2 (P2) and adult rats by dissecting the ON from an intact retina. Retinal astrocytes were isolated from P2 rats. Cells were used between passage 3-6.
RGC Polarization Assays: Astrocytes were suspended in matrigel and printed using an Allevi 3 bioprinter at the center of radial electrospun scaffolds. RGCs were seeded the following day and cultured for 2 days before fixing. Samples were analyzed for the direction the RGCs extend their axon.
Astrocyte and ECFC Migration Assays: RGCs were seeded on half of the radial scaffold and P2 ONHA alone or with endothelial colony forming cells (ECFC) were positioned at the scaffold center. Samples were cultured for 14 days, fixed and stained. Samples were analyzed for the percentage of cells migrating on the RGC half of the scaffold compared to those on the fibers alone half.
Results: RGCs seeded on scaffolds with developmental P2 ONHA were observed to have an increased polarization of axon growth towards the scaffold center where as RGCs on scaffolds with adult ONHA, P2 retinal astrocytes or two cortical astrocyte cell lines derived from P2 mice showed no change in polarization compared to matrigel printed alone. A higher percentage of ONHA were observed to migrate on the half of the scaffold seeded with RGCs with those ONHA migrating a further distance as well. Similarly, ECFCs were observed to preferentially migrate on the RGC half of the

scaffold, however while some ECFCs were observed to migrate on the non-RGC half of the scaffold, vascular tubes were only observed to form on the half of the scaffold which contained RGCs.

Conclusions: RGC guidance and vascularization in vivo both require the presence of ONH glia and vascular endothelial cells, both of which are absent in the self-forming RO. In this study, we demonstrate that introducing astrocytes from a specific developmental time point via 3D printing in combination with electrospun scaffolds are able to polarize RGC growth such that it more closely matches that found in vivo while also demonstrating that these three cell types recapitulate the mechanism for retinal vascularization. These results suggest a method for forming RO that can better be used for studying glaucoma while also demonstrating a potential method for vascularizing CNS tissue engineered constructs.

PLATFORM SESSION II

CHAIR: DR. MACHELLE PARDUE

11-12 PM

Detection and Treatment of Early-stage Diabetic Retinopathy

Pardue, Machelle[1]
[1]EMORY UNIVERSITY, ATLANTA, GEORGIA

Purpose: Diabetic retinopathy is clinically diagnosed by the presence of retinal vascular pathology which typically takes ~10 years to develop after diabetes onset. Recent evidence from animal models and patients with diabetes shows retinal neuronal changes with diabetes, prior to vascular pathology. We investigated a non-invasive functional biomarker for earlier detection of diabetic retinopathy.

Methods: Retinal function was longitudinally measured in STZ-induced diabetic rodents using electroretinogram (ERG) in response to a range of dark-adapted stimuli. Diabetic rodents were treated with levodopa. A clinical trial was performed with individuals having diabetes and no retinopathy, as assessed by fundus photographs. Individuals with ERG delays in response to dim flash were treated for two weeks with sustained release levodopa and followed for 4 weeks.
Results: Diabetic rodents showed delayed implicit times for ERG oscillatory potentials (OPs) in response to dim stimuli. Levodopa treatment preserved retinal function. In human participants, half of the individuals with diabetes and no retinopathy had delayed OPs and levodopa treatment restored function to non-diabetic control values.
Conclusions: ERG recordings in response to dim flash stimuli revealed early changes in rod pathway function that precede vascular defects in the fundus in both pre-clinical and clinical studies. These results support the use of the dim flash OP response as a functional biomarker for early stage diabetic retinopathy and provide evidence that the levodopa can be protective for this early retinal dysfunction.

Elevated IOP facilitates T cell mediated RGC loss

Mayberry, Jordan[1,2,3]; Xian, Shuyu[1,2]; Kuehn, Markus, H.[1,2,3]
[1]DEPARTMENT OF OPHTHALMOLOGY AND VISUAL SCIENCES, UNIVERSITY OF IOWA; [2]VA CENTER FOR THE PREVENTION AND TREATMENT OF VISUAL LOSS, IOWA CITY; [3]INTERDISCIPLINARY GRADUATE PROGRAM IN GENETICS, UNIVERSITY OF IOWA

Purpose: Glaucoma is a neurodegenerative disease that causes loss of retinal ganglion cells (RGC), and subsequently, loss of vision. Previous studies in our lab have demonstrated that adoptive transfer of CD3+ cells from animals with glaucoma into healthy recipients results in progressive loss of RGC, suggesting a functional role of adaptive immunity in the pathology of the disease. A crucial first step in this process is the extravasation of T-cells into the immune-privileged retina. This study was conducted to determine if elevated IOP facilitates extravasation using donor mice with a fixed T-cell receptor against GFP (Just EGFP Death Inducing, JEDI) and Thy1-GFP+ recipient mice that exhibit GFP expression in a subset of RGC.
Methods: Mild IOP elevation was induced in the eyes of Thy1-GFP+ mice through intracameral injection of Ad5.Myoc^{Y437H} (n=12). Thy1-GFP+ and Thy1-GFP- control animals received intracameral injections of Ad5.empty (n=11-12/per group). Following injection, IOP was monitored by rebound tonometry and GFP positive cells were imaged by fundoscopy for 7 weeks. 4 weeks following injection, spleens from donor JEDI mice were harvested, homogenized, and transferred into recipient Thy1-GFP mice. At week 4.5 following intracameral injection, one group received an intraperitoneal injection of lipopolysaccharide (LPS). At the conclusion of the experiment, retinas were collected, whole-mounted, stained using antibodies directed against CD3+ T cells, and visualized using confocal and widefield fluorescence microscopy.
Results: Following intracameral injection and adoptive transfer of JEDI splenocytes, all mice experienced some degree of RGC loss on fundus imaging. Losses are similar in mice that did not receive splenocytes (-4.14%), those that did receive splenocytes but without elevated IOP (-2.83%), and those receiving splenocytes and LPS (-4.5%). In contrast, mice receiving splenocytes and experienced elevated IOP lost significantly more RGC (-18.43%, p=0.0062). In these animals RGC loss was particularly noticeable in the central retina rather than in the periphery. Detectable levels of retinal CD3+ cells were low and similar in all groups.
Conclusions: Slight elevation in IOP facilitates T cell mediated degradation of RGC. LPS-mediated weakening of the blood-retina barrier by itself is insufficient to achieve RGC loss, suggesting that additional IOP-mediated mechanisms are required to overcome retinal immune privilege.

Interferon Regulatory Factor 1 (IRF1) in Retinal Neurodegeneration

Huang, Hu[1]; Jeevan, Varun[1]; Rajooldezfuly, Ali[1]; Mays, Logan[1]; Elahi, Fazle[1]
[1]DEPARTMENT OF OPHTHALMOLOGY, THE UNIVERSITY OF MISSOURI, SCHOOL OF MEDICINE

Purpose: Retinal neurodegeneration associated with neuroinflammation occurs in various retinal diseases, including age-related macular degeneration (AMD), retinitis pigmentosa (RP), diabetic retinopathy (DR), and glaucoma. This study aims to investigate the role and mechanism of interferon regulatory factor 1 (IRF1) in retinal neurodegeneration. Although IRF1 is a well-characterized inflammatory regulator, whether and how it is involved in the development of retinal neurodegeneration has not been explored.
Methods: scRNA-sequencing and QRT-PCR analyses determine gene expression in retinal cells. Immunofluorescence staining and immunoblotting examine protein expression. The animal models of retinal neurodegeneration model were generated using the chemicals sodium iodate and NMDA. Spectral domain-optical coherence tomography (SD-OCT) and fundus imaging with Micron OCT system (Phoenix) examine retinal structures of Irf1 gene knockout mice vs. C57 wild-type control mice. ERG determines the retinal visual function. PI stain and TUNEL Assay quantifies apoptotic cell death. SeaHorse XF96 Mito Stress test examines the mitochondrial metabolic function of retinal explants. Statistical analysis was performed using GraphPad Prism and Excel.
Results: IRF1 is primarily expressed by glial cells in the retina, such as Müller cells and microglia. IRF1 acts as a stress sensor, and its expression levels are upregulated in retinal tissues and glial cells under various stress conditions, such as IFNγ/TNFα, LPS, H2O2, and NMDA. Genetic deletion of the IRF1 gene in mice protects against retinal degeneration (e.g., photoreceptors) induced by the oxidizing agent sodium iodate. Additionally, IRF1 gene knockdown via siRNA reduces neuroinflammation related to microglial activation in the retina. IRF1 siRNA prevents NMDA-induced retinal ganglion cell (RGC) loss and visual dysfunction. Mechanistically, IRF1 mutation or knockout prevents microglia from shifting from a homeostatic state to an activated one both in vivo and in vitro. IRF1 mutation affects mitochondrial metabolic function and gene expression. Conditioned culture medium (CM) from IRF1-mutant microglia reduces retinal cell death compared to CM from IRF1 wild-type microglial cells.
Conclusions: IRF1 contributes to retinal neurodegeneration by regulating microglia-mediated neuroinflammation and mitochondrial metabolic function.

Neurofilament light chain as a biomarker of neurodegeneration in feline glaucoma

McLellan, Gillian[1]; Adams, Grace[1]; Kiland, Julie[1]; Oikawa, Kazuya[1]
[1]UNIVERSITY OF WISCONSIN-MADISON

Purpose: Glaucoma is a leading cause of blindness worldwide, characterized by degeneration of retinal ganglion cell (RGC) somas and their axons in the retinal nerve fiber layer (RNFL) and optic nerve (ON). Neurofilament light chain (Nfl) is a neuronal cytoplasmic protein that is highly expressed in myelinated axons and has been proposed as a plasma biomarker in various neurodegenerative diseases in people and animals, but sparse studies in glaucoma patients have yielded conflicting results. Our goal was to determine association between circulating Nfl concentration and structural and functional markers of ON degeneration in cats with glaucoma.
Methods: Biobanked serum and plasma samples from 43 young adult cats with feline congenital glaucoma (FCG) due to LTBP2 mutation and 15 normal control cats of both sexes were assayed for Nfl (Quanterix Single molecule array [Simoa]; HD-X analyzer, [Quanterix, Billerica, MA]). Optical coherence tomography (OCT) imaging and ON axon count data provided in vivo and direct histologic ON structural metrics, respectively. Visual evoked potentials (VEP) provided a measure of ON function. Longitudinal intraocular pressure (IOP) data were also recorded. Optical coherence tomography (OCT; Heidelberg Spectralis) imaging and ON axon count data provided in vivo (RNFL thickness) and direct histologic ON structural metrics, respectively. Visual evoked potentials (VEP) provided a measure of ON function. Longitudinal intraocular pressure (IOP) data were also available for each cat.
Results: Serum Nfl concentrations were significantly higher in FCG than in normal cats ($p=0.0009$), with no significant difference between sexes, and no association with age in this young cohort. Serum Nfl concentration showed a weak negative correlation with ON axon count and VEP amplitude (Pearson $r = -0.213$ and -0.219, respectively) and modest positive correlation with VEP latency ($r=0.286$ and maximum IOP value measured in the worst affected eye ($r= 0.348$) Serum and plasma Nfl values were highly correlated ($r^2 =0.996$) and could be interchangeable in a clinical setting.
Conclusions: Circulating serum Nfl concentrations are elevated in glaucoma but are only modestly associated with direct measures of ON neurodegeneration in this feline glaucoma model, which may limit clinical application as a fluid biomarker in this disease.

PLATFORM SESSION III

CHAIR: DR. ELLIOTT SOHN

1-2 PM

Integrated Systemic Inflammation and AI-Driven OCT Metrics in Neovascular Age-Related Macular Degeneration: Bridging Clinical Correlations and Pathophysiology

Sohn, Elliott[1]; Zacharias, Zeb[1]; Lee, Kyungmoo[1]; Bach, Bernardo; Chava, Srinivas[1]; Wang, Kai[1]; Wu, Amy[1]; Mukhopadhyay, Chirantan[1]; Coussa, Razek[1]; Han, Ian[1]; Scheetz, Todd[1]; Fingert, John[1]; Abramoff, Michael[1]; Stone, Edwin[1]; Houtman, Jon[1]; Sonka, Milan[1]
[1]UNIVERSITY OF IOWA

Purpose: Neovascular age-related macular degeneration (nvAMD) has a complex pathogenesis, with genetics (e.g. CFH, ARMS2, and MMP9) and inflammation playing key roles. Artificial intelligence (AI) has enabled unprecedented analyses of fluid in optical coherence tomography (OCT) imaging. We determined the association of systemic inflammatory markers with visual acuity and fluid parameters derived from AI based quantitative OCT analysis of subjects with nvAMD.
Methods: An ensemble approach of U-Net architectures (convolutional neural networks) developed for retinal pathology segmentation, was trained to simultaneously segment intraretinal (IRF), subretinal fluid (SRF), and pigment epithelial detachment (PED) regions from Heidelberg OCT scans in nvAMD subjects (n=100) receiving consecutive anti-VEGF injections (comprising 6320 OCTs). 10-fold cross-validation was performed with training OCT scans from 90 subjects and a validation dataset of 10 subjects.
An optimized spectral flow cytometry panels using Cytek Aurora assessed the steady-state phenotype (31-colors) and effector functions (23-colors) of human T cells from PBMCs in a subset of nvAMD subjects previously genotyped for MMP9. Using our 31-color steady-state panel, we determined the subset distributions of the main T cell populations (e.g., naïve, memory, etc.) in these subjects.
Results: 10-fold cross-validation yielded high R^2 of 0.85, 0.95, and 0.89 for predicted vs ground truth of OCT quantified IRF, SRF, and PED volumes, respectively. Flow cytometry showed that patients with high-risk MMP9 genotype have CD4 T cells with a trend (p=0.051) toward greater capacity to generate IL-4 and IL-13, both of which are major contributors to fibrosis. In nvAMD subjects receiving intravitreal anti-VEGF injections, a significant correlation of worse VA at initial, 2 years, and final visit was observed with several subsets of T cells. %CD4 Tem early-like cells showed a positive association with PED volume at subjects' initial visit (p=0.0066).
Conclusions: A validated AI-based image analysis algorithm in nvAMD reveals association of worse fluid with higher systemic levels of inflammation. Subjects with worse visual acuity in nvAMD may experience increased levels of inflammation. Patients with high risk MMP9 genotype may be skewed to a Th2 phenotype that could be associated with dysregulated wound healing in nvAMD.

Nystagmus recordings and correlation with underlying diagnosis in children

Davis, Kristin[1]; Dumitrescu, Alina[1]; Vandelune, Joel[1]; Peotta-Jacobsen, Veronica[1]
[1]DEPARTMENT OF OPHTHALMOLOGY AND VISUAL SCIENCES, UNIVERSITY OF IOWA

Purpose: Nystagmus is uncontrolled, repetitive movements of the eyes. Nystagmus is a clinical symptom associated with a multitude of diseases ranging from neurological causes, such as periventricular leukomalacia or congenital malformations of the brain, to ophthalmic etiologies such as albinism, retinal diseases with onset in childhood, to benign causes. Nystagmus can be clinically described based on its amplitude, frequency, direction of oscillations, and variation with the gaze direction. The diagnostic workup to identify the cause of nystagmus is inclusive and targeted simultaneously, including imaging, electrophysiology, and genetic testing. Despite extensive work done to classify nystagmus, an objective method has yet to be. This study aims to evaluate eye movement recordings' role in the correct and complete diagnosis of patients with nystagmus.
Methods: Patients with nystagmus evaluated in the pediatric ophthalmology and pediatric inherited eye disorder clinics at the University of Iowa were prospectively enrolled in this study. In addition to their regular workup, patients underwent video recording of their nystagmus using the Neurolign Dx 100. This device is an FDA-cleared eye-tracking device previously used for other eye conditions and is currently under investigational use for recording nystagmus. It consists of VR-style goggles worn by the patient. Horizontally and vertically moving targets are displayed, while multiple infrared cameras record the eye movements and oscillations. This painless, noninvasive procedure does not require anesthesia and roughly takes 10 minutes. The recordings are then analyzed to measure nystagmus direction, frequency, amplitude, intensity, and waveform morphology. The nystagmus characteristics will then be compared with the etiology as determined by the clinical workup for correlation.
Results: Thus far, our study has enrolled 40 patients: 22 males and 18 females between the ages of 4 and 18. Nystagmus recordings were successfully obtained from all patients. Ten individual waveform morphology patterns were identified. The classification and waveform morphology analysis obtained using automatic eye-tracking recordings was more detailed and reproducible than the clinical description. Four different categories of underlying nystagmus etiology were identified. Of those enrolled, 45% had oculocutaneous albinism, 22.5% had optic nerve anomalies, 20% had retinal dystrophies, and 12.5% had congenital motor nystagmus. Visual acuity was grouped into 3 categories: good (20/40 or better), moderately decreased (20/40 to 20/100), and poor (worse than 20/100). The underlying etiology correlated with the severity of visual acuity. However, no correlation between the nystagmus waveform

morphology or pattern and the underlying diagnosis or visual acuity has been established thus far.

Rho-kinase inhibitor nanomedicine for Fuchs endothelial corneal dystrophy

Dusane, Apurva[1]; Skeie, Jessica M.[2]; Shevalye, Hanna[3]; Eggleston,Tim[3]; Kalidindhi,Swathi[1]; Okafor, Cynthia[1]; Schmidt, Gregory A.[3]; Salem, Aliasger K.[1]; Greiner, Mark A.[2]

DEPARTMENTS OF [1]PHARMACEUTICAL SCIENCES AND EXPERIMENTAL THERAPEUTICS; [2]OPHTHALMOLOGY AND VISUAL SCIENCES, UNIVERSITY OF IOWA; [3]IOWA LIONS EYE BANK

Purpose: Currently, surgical procedures are the only first line treatment for Fuchs endothelial corneal dystrophy (FECD). We aimed to develop a rho-kinase inhibitor nanomedicine approach for targeting corneal endothelial cells (CECs) to prevent FECD progression. Rho-kinase inhibitor comprising nanoparticulate eye drops were used.
Methods: Nanoparticles were characterized by measuring size and charge using Zetasizer. Drug loading and encapsulation efficiency were quantified using HPLC-DAD. Particles were characterized using XRD. Formulation stability was examined for 2 months at 4°C and 25°C. Release studies were performed at 37°C. Safety and Efficacy of formulation was evaluated on mice with a well-characterized FECD mutation (129S6/SvEvTac Col8a2Q455K). Optical coherence tomography (OCT) and confocal imaging were performed to study progression of guttae and changes in CEC morphology. An initial baseline for these techniques on a mouse cohort was recorded at the age of 4 months, then subsequently after 1 month of therapy (age of 5 months) and after 2 months of therapy (age of 6 months).
Results: Particle size was determined to be 81.60± 2.48 nm with a narrow polydispersity index (PDI) (n = 3 batches). XRD indicated drug incorporation in nanoparticles. Formulation exhibited in vitro controlled drug release. In the mouse model, the nanoparticulate drug therapy had a good safety profile and low off-target side-effects. CCM indicated an overall improvement in cell health with RKI nanoparticle treatment.
Conclusions: Findings indicate the successful development of rho-kinase targeted nanoparticles as a possible drug delivery strategy for CECs to prevent FECD progression.

A candidate gene therapy vector restores cone function in a mouse model of Bardet-Biedl Syndrome Type 10

Drack, Arlene V.[1]; Lobeck, Brianna[1]; Rankin, Tyler[1]; Stanley, Sarah; Kalmanek, Emily[1]; Anastasios, Georgiadis[2]; Holthaus, Sophia[2]; Thompson, Jacob[1]; Hassan, Salma[1]; Sheffield, Val[3]; Hsu, Ying[1]

[1]DEPARTMENT OF OPHTHALMOLOGY AND VISUAL SCIENCE, UNIVERSITY OF IOWA; [2]MEIRAGTX; [3]DEPARTMENT OF PEDIATRICS, UNIVERSITY OF IOWA

Purpose: Biallelic mutations in BBS10 are the second most common cause of Bardet Biedl Syndrome (BBS), a ciliopathy associated with blindness in early life as well as obesity, polydactyly, and renal failure. Subretinal gene therapy using an AAV2/Anc80 capsid carrying the mouse Bbs10 gene showed efficacy in a BBS10 knock out mouse model. AAV8-RK-hBBS10 and AAV8-CAG-hBBS10, developed for clinical translation, were used to treat the same mouse model to assess safety and efficacy.
Methods: The human BBS10 gene driven by either the rhodopsin kinase (RK) promoter or the CMV enhancer/chicken β-actin promoter (CAG) was cloned into a shuttle plasmid, packaged into AAV2/8, and titered by quantitative PCR for in vivo gene delivery. For testing the safety of these candidate vectors in mice, 2.48×10^{11} vg of either vector was subretinally injected into unaffected wild-type (WT) or heterozygous (HET) mice in a 2 μL volume, and optical coherence tomography (OCT) was performed at 1-, 3-, and 5-months post injection (MPI). A lead candidate vector was selected. For evaluating efficacy, Bbs10-/- mice were subretinally injected with 2.48×10^{9}, 2.48×10^{10}, or 2.48×10^{11} vg/eye of the lead candidate vector, and outcome measures including electroretinography (ERG) and OCT were conducted at 1-, 2-, 3-, 5-, and 7-MPI. A visually-guided swim assay for evaluating rodent functional vision was conducted at 5- to 7-MPI.
Results: Unaffected HET or WT mice receiving 2.48×10^{11} vg/eye of AAV8-CAG-hBBS10 demonstrated retinal atrophy on OCT. Those receiving AAV8-RK-hBBS10 did not, and AAV8-RK-hBBS10 was designated the lead candidate vector. Subretinal injections of AAV8-RK-hBBS10 were performed in Bbs10-/- mice at 2.48×10^{9}, 2.48×10^{10}, or 2.48×10^{11} vg/eye. Eyes receiving subretinal gene therapy developed a light adapted (LA) 5 Hz flicker ERG cone response, while no untreated eyes had a recordable LA 5 Hz flicker cone ERG. Eyes treated with high dose had higher average cone ERG amplitudes (17.8 ± 5.3 μV) compared to eyes that received the medium dose (9.6 ± 1.1 μV, p = 0.086), low dose (5.6 ± 1.0 μV, p ± 0.001), or no treatment (5.0 ± 0.7 μV, p ± 0.0001) at 5-MPI (averages ± SEM,two-way ANOVA, post-hoc Tukey's multiple comparisons test). Treated Bbs10-/- mice averaged less time to find the platform in a visually-guided swim assay compared to untreated with no significant difference in times between different doses.

Conclusions: The choice of promoter to drive gene expression impacts safety profiles of the vector for in vivo gene delivery. AAV8-RK-hBBS10 had a superior safety profile and showed remarkable efficacy in vivo, causing eyes with no recordable cone ERG to develop one, and maintain it for at least 5 months after treatment. Mice with BBS10 retinal degeneration treated with high dose AAV8-RK-hBBS10 had higher ERG cone amplitudes and better functional vision compared to untreated BBS10 mice.

POSTER PRESENTATIONS

EVEN NUMBERED POSTERS : PRESENTING 10-11 AM

ODD NUMBERED POSTERS: PRESENTING 2-3 PM

Author	Poster Number	Title
Aditya Somisetty	1	Comparing the effects of two rho-kinase inhibitors, ripasudil and netarsudil, for the treatment of Fuchs endothelial corneal dystrophy
Emma Hartness	2	A Clinical Challenge: Delayed Diagnosis of Autoimmune Polyglandular Syndrome Type II in a Patient with Thyroid Eye Disease
Kaitlyn Grimes	3	Risk of vision threatening complications with sports in Juvenile X-linked Retinoschisis
Merri-Grace Jones	4	Characterization of immune populations in cervical lymph nodes in Ad5 viral vector model of glaucoma
Maram El-Geneidy	5	The Educational Efficacy of a Novel Interactive, Case-Based Educational Module for Prescribing Pediatric Spectacles
Julian Pablo	6	Implementation of a Mediterranean Diet Exacerbates Visual and Motor-Sensory Impairment in an Animal Model of Multiple Sclerosis
Tyler Rankin	7	N-Acetylcysteine ameliorates loss of the electroretinogram b-wave in a Bardet-Biedl Syndrome Type 10 mouse model
Matt Harper	8	Enhanced attention in rats following blast-induced traumatic brain injury
Brianna Lobeck	9	Evaluating efficacy of subretinal gene therapy in moderately advanced retinal degeneration using a mouse model of Bardet-Biedl Syndrome type 10
Hend Al-Kaylani	10	Comparison of trabeculectomy approach on surgically-induced astigmatism in the treatment of open-angle glaucoma
Sarah Stanley	11	Diurnal Variation Contributes to Cyst Severity in a Mouse Model of X-Linked Juvenile Retinoschisis
Khyathi Thallapureddy	12	Astrocyte Origin and Developmental Stage Influences Ability to Guide Retinal Ganglion Cell Outgrowth

Sheila Baker	13	Mechanisms of Kv2.1/Kv8.2 associated vision loss.
Noriyoshi Takahashi	14	Dynamic Retinal Blood Flow Analysis: Heartbeat-Correlated Artery and Vein Identification in Laser Speckle Flowgraphy
Ram Sibani	15	Analyzing Palpebral Fissure Width to Brow Fat Span Ratio in Female Celebrities
Lydia Yang	16	Improvements in Documentation and Billing of Ophthalmology Diagnostic Testing
Lindsay Chun	17	Orbital Echographic Assessment of Extraocular Muscle Size Changes associated with Teprotumumab
Simon Kaja	18	Particulate Matter Elicits Ocular Toxicity Through Multiple Distinct Cellular and Biochemical Pathways
Matthew Van Hook	19	Effects of ocular blast pressure injury on neurons and glia in the visual thalamus
Bhuvanachandra Pasupuleti	20	Novel approaches to evaluating drug candidates to mitigate antibody-drug conjugate chemotherapy associated corneal toxicity
Michael Hayes	21	A Clinical Trial in a Dish to Investigate Neuroprotection and Remyelination in Optic Neuropathies
Maria F. Valdes Michel	22	Changes in Spinal Cord Gene Expression correlates with Motor-Sensory Impairment in an EAE Model of Optic Neuritis
Jui-Kai Wang	23	Beyond Retinal Thickness: Textural Changes Precede Retinal Thinning in Acute Non-Arteritic Ischemic Optic Neuropathy (NAION)
Jennie C Smith	24	Knockout of component C3 in complement cascade decreases photoreceptor function without concomitant cell loss
Candace Betancourt-Szymanowska	25	Endothelin-1 overexpression elicits cellular elastinopathy and reactive astrocytosis in rat optic nerve head astrocytes
Shaylah McCool	26	Microglia Polarization in the Visual Thalamus of an AD Mouse Model
Joshua Luscan	27	Nicotinamide Induces Cobblestone Morphology in ARPE-19 Cells but Not in Primary RPE Cells: A Comparative Study
Jeffrey Anders	28	A Longitudinal Analysis of a MOG Induced Mouse Model of Multiple Sclerosis-like Optic Neuritis
Varun Shravah	29	Evaluation of the Choroidal Sprouting Assay as an Ex Vivo Model of Microvascular Angiogenesis
Erin Capper	30	Histopathologic Evaluation of Human Optic Nerves in Multiple Sclerosis Patients

Ying Hsu	31	A deep learning model for evaluating fundus image quality in the UK Biobank dataset
Ben Kragerud	32	Microbead-Induced Ocular Hypertension and Optic Nerve Crush Reduce Ganglion Cell Complex Thickness in a Mouse Model of Alzheimer's Disease
Lori Wallrath	33	N-acetylcysteine (NAC) suppresses retinal defects in Drosophila models of SNRNP200-associated Retinitis Pigmentosa
Edward Linton	34	Laser Speckle Flowgraphy Reveals Widespread Reductions in Ocular Blood Flow in Non-exudative Age-related Macular Degeneration Independent of Ocular Perfusion Pressure

Poster #1

Comparing the effects of two rho-kinase inhibitors, ripasudil and netarsudil, for the treatment of Fuchs endothelial corneal dystrophy

Somisetty, Aditya[1]; Shevalye, Hanna[2]; Eggleston, Timothy[2]; Skeie, Jessica[1]; Dusane, Apurva[1]; Gregory, Schmidt[2]; Salem, Aliasger[2]; Greiner, Mark[1]
[1]University of Iowa Carver College of Medicine; [2]Lions Eye Bank, Iowa City, IA

Purpose: Fuchs endothelial corneal dystrophy (FECD) is characterized by a progressive decline in corneal endothelial cell (CEC) health, resulting in corneal edema and gradual vision loss. FECD affects approximately 300 million people globally and is the leading indication for keratoplasty. The only current therapy is corneal transplantation. Therefore, research has recently been focused on developing topical treatments which promote the growth and migration of functional CECs in FECD patients undergoing DSO therapy, as well as to prevent FECD from progressing to transplant need to begin with. Rho-kinase inhibitors (RKIs), such as netarsudil (FDA approved) and ripasudil (approved in Japan) can enhance CEC density, promote cell migration, improve intercellular adhesion, and suppress apoptosis. A comparative study of netarsudil and ripasudil could provide key insights into netarsudil's feasibility and noninferiority for a crucially needed FECD treatment in the US.
Methods: Efficacy of both netarsudil and ripasudil were quantified in gap closure assays using FECD and non-FECD cell cultures. Briefly, CECs were grown to confluency in gap assay chambers. When confluent, the chambers were removed, leaving a defined gap. At time=0, CECs were treated with netarsudil or ripasudil and imaged every 8 hours for a total of 72 hours. Gap closure and CEC proliferation were quantified using ImageJ software. Netarsudil and ripasudil were also compared in a well-characterized mouse model (COL8A2Q455K) for preventing FECD progression. Briefly, mice were treated daily for two months beginning at 3 months of age (when FECD disease is present). Enucleated eyes were labeled with tight junction protein ZO-1 and imaged using confocal microscopy. Images were used for morphometric analysis of CEC areas, CEC density, hexagonality, and multinucleation, which are key indicators of CEC function. Statistical analysis was performed using Student’s t-test between groups.
Results: After conducting multiple trials of gap closure assays on FECD and non-FECD cell cultures at various concentrations, we found that netarsudil was not noninferior to ripasudil in promoting CECs to reach full confluency. We are currently finalizing the collection of morphometric data, which is crucial for assessing the detailed cellular effects of these treatments. This analysis will determine whether netarsudil is noninferior to ripasudil in preventing the development of FECD-like phenotypes, characterized by increased CEC area, decreased CEC density, reduced hexagonality, and increased multinucleation. These metrics are key indicators of cellular health and function in

FECD, and their evaluation will provide comprehensive insights into the efficacy of netarsudil compared to ripasudil.
Conclusions: Although netarsudil did not show noninferiority to ripasudil in the initial gap closure assays, the ongoing collection of morphometric data is essential for a comprehensive assessment. Understanding the potential of netarsudil in preventing FECD progression could lead to new therapeutic options.

Poster #3

Risk of vision threatening complications with sports in Juvenile X-linked Retinoschisis

Grimes, Kaitlyn[1]; Steurnagel Del Valle, Guilia[1]; Peotta-Jacobsen, Veronica [1]; Dumitrescu, Alina[1]; Arlene, Drack[1]

[1] Department of Ophthalmology and Visual Sciences, University of Iowa

Purpose: Juvenile X-Linked Retinoschisis (XLRS) is a hereditary disease resulting from a mutation in the RS1 gene, which encodes retinoschisin, a protein necessary for cell adhesion and structure in the retina. Decreased adhesion between the retinal layers puts these patients at higher risk for ocular complications such as retinal detachment, vitreous hemorrhage, and posterior vitreous detachment due to ocular trauma. These ocular complications can result in significant vision loss or blindness, which is why many ophthalmologists recommend that patients with XLRS avoid contact sports. Currently, there is a scarcity of literature regarding the risks of ocular complications associated with playing contact sports, with or without protective eyewear, for patients with XLRS. The purpose of this study is to provide additional evidence on the risks of sports-related ocular complications in patients with XLRS to help assist families and clinicians make informed decisions about sports participation.

Methods: This study retrospectively analyzed chart records of patients with a diagnosis of XLRS. The study was approved by the Institutional Review Board of The University of Iowa and was conducted in accordance with the tenets of the Declaration of Helsinki on Biomedical Research Involving Human Subjects. This study included 56 male patients that were seen at the University of Iowa Department of Ophthalmology, a tertiary referral center, between January 1, 2000, and January 30, 2023. All patients had a clinical diagnosis of XLRS made by an Inherited Retinal Disorders specialist based on ocular findings associated with family history consistent with X-linked inheritance, and/or the presence of pathogenic or likely pathogenic variants in the RS1 gene.

Results: Of 56 patients, 24 patients participated in a variety of contact and non-contact sports. 32 patients were counseled about the risk of involvement in sports and 28 were additionally prescribed or advised to wear safety goggles. Of the 24 patients in sports, 9 wear safety goggles, 12 do not wear safety goggles, and 3 had unknown compliance. Only 1 patient experienced sports related ocular trauma that led to a complication, a posterior vitreous detachment. Among the 24 subjects who participated in sports, 2 retinal detachments were diagnosed, but none of those were related to ocular trauma during those activities. Of the 32 patients that did not participate in sports, 6 retinal detachments occurred. There was no significant association between the practice of sports and the occurrence of retinal detachments. Our results show that participation in

sports did not significantly impact the final best-corrected visual acuity, refraction, or central macular thickness in patients with XLRS.
Conclusions: This study provides valuable insights into the risks associated with sports participation in patients with XLRS and the potential benefits of wearing protective eyewear. Our study analysis revealed that there was no significant difference in the occurrence of retinal detachments between patients who participated in sports and those who did not. Although two retinal detachments occurred in the sports-participating group, there was no evidence of those being related to ocular trauma, and the overall incidence was not higher compared to those who did not participate in sports. Additionally, all patients who experienced ocular injuries associated with the practice of sports were not wearing protection eyewear at the time of trauma. While the risk of serious complications exists, it may not be as prevalent or directly correlated to sports activities, especially when protective measures are taken.

Poster #5

The Educational Efficacy of a Novel Interactive, Case-Based Educational Module for Prescribing Pediatric Spectacles

El-Geneidy, Maram[1]; Strampe, Margaret[1]; Johnson, Brett [1]; Kemp, Pavlina [1]; Andrew, Pouw[1]
[1] Department of Ophthalmology and Visual Sciences, University of Iowa

Purpose: The purpose of this study is to evaluate the teaching effectiveness of a novel, web-based-interactive educational module on medical decision-making for pediatric spectacle correction by comparing pre-test and post-test performance of medical students and ophthalmology resident learners.
Methods: The module was created using a series of cases and multiple-choice questions. Students and residents completed a pre-test of the questions without explanations prior to completing the module. The module provided explanations after each question. A post-test was completed following completion of the module. Pre-test and post-tests were taken within 1 week of each other and results were compared. Questions were tagged by topic to allow for comparison and evaluation of specific sub-topics. Sub-topics tested included emmetropia, mixed anisometropia, physiologic astigmatism for age, accommodative esotropia, high myopia, meridonial emblyopia, refractive amblyopia, anisometropia, hyperopia, myopic anismetropia, regular astigmatism, asthenopia, hyperopic astigmatism, physiologic range hyperopia, astigmatism, latent hyperopia, and physiologic range refractive error.
Results: 12 medical students and 8 ophthalmology residents completed the module. All participants demonstrated a score increase on the post-test compared to the pre-test. Among the sub-topics tested, resident learners demonstrated the greatest score increase in hyperopia 33% (±25%), followed by emmetropia 25% (SD ± 27%) and regular astigmatism 22% (±16%) respectively. Medical student learners demonstrated the greatest score increase in emmetropia 46% (±33%), physiologic range refractive error 40% (±32%), and physiologic range hyperopia 32% (±26%), respectively.
Conclusions: Overall, all participants demonstrated improved knowledge scores after taking the educational module. This novel interactive module may be effective for educating ophthalmic trainees on prescription practices for pediatric spectacles.

Poster #7

N-Acetylcysteine ameliorates loss of the electroretinogram b-wave in a Bardet-Biedl Syndrome Type 10 mouse model

Rankin, Tyler[1]; Mayer, Sara[1]; Laird, Joseph[1]; Brianna, Lobeck[1]; Kalmanak, Emily[1]; Drack, Arlene[1]

[1] Department of Ophthalmology and Visual Sciences, University of Iowa

Purpose: Bardet Biedl Syndrome (BBS) is a ciliopathy which leads to early photoreceptor cell death and blindness. Natural history of the electroretinogram (ERG) demonstrates a no b-wave phenotype around 4 months of age, which evolves to a nonrecordable ERG. The purpose of this study is to determine whether N-Acetylcysteine (NAC) ameliorates the no b-wave (nob) ERG phenotype in the Bbs10-/- mouse.

Methods: Nine Bbs10-/- mice (18 eyes) were provided with NAC dissolved in water at a concentration of 7 mg/mL and pH of 4 for 4 months. Nine Bbs10-/- mice (18 eyes) were provided plain water, 9 Bbs10+/-unaffected control mice (18 eyes) were maintained on NAC, with an additional 6 Bbs10+/-control mice (12 eyes) were maintained on plain water. ERGs were conducted monthly in dark, and light adapted conditions. Outer nuclear layer (ONL) thickness was measured monthly using optical coherence tomography. Immunohistochemistry was performed to observe synaptic localization within the retina.

Results: NAC supplementation significantly ameliorated the progressive degeneration of the ONL in Bbs10-/- mice ($p = 0.0002$; Bonferroni one-way ANOVA). No statistical difference was observed in a-waves between water and NAC-treated mice. However, NAC-treated mice had significantly higher b-wave amplitudes compared to water-treated Bbs10-/- mice (Welch's t-test, $p = 0.0029$). Typically, a- and b- waves are proportionally correlated, but this was not observed in Bbs10-/- mice at four months old due to a no b-wave phenotype. NAC treatment restored this proportionality, as indicated by a significant recovery in b-wave ($p = 0.0036$; simple regression model). Additionally, immunohistochemistry revealed synapse retraction into the ONL in Bbs10-/- retinas lacking a b-wave, with a statistically greater number of synapses in the ONL compared to those with a recordable b-wave ($p = 0.0492$; ordinary one-way ANOVA).

Conclusions:These findings suggest NAC as a promising therapeutic intervention for managing BBS10-related retinal degeneration; ERG suggests NAC supports retinal synaptic function.

Poster #9

Evaluating efficacy of subretinal gene therapy in moderately advanced retinal degeneration using a mouse model of Bardet-Biedl Syndrome type 10

Lobeck, Brianna[1]; Hsu, Ying[1]; Stanley, Sarah[1]; Kalmanek, Emily[1]; Ethan, Brandauer[1]; Drack, Arlene[1]

[1] Department of Ophthalmology and Visual Sciences, University of Iowa

Purpose: Bardet-Biedl Syndrome (BBS) is a rare inherited pleiotropic disease with at least 28 causative genes known to date. Bardet-Biedl Syndrome type 10 (BBS10), a prevalent sub-type of BBS, is caused by mutations in the BBS10 gene. Retinal degeneration leading to severe vision loss is one of the most penetrant phenotypes. Studies have shown promise in delaying photoreceptor cell loss by treating Bbs10-/- mice with subretinal gene augmentation therapy for BBS10 between P23-P30. Mice at this age displayed mild degeneration compared to heterozygous or wild-type mice. The average age of diagnosis for BBS-associated retinal degeneration is 5-12 years, when visual impairment and significant retinal degeneration become noticeable. It is unknown whether subretinal gene therapy can delay further photoreceptor cell loss in patients with moderately advanced retinal degeneration. This study examines the efficacy of subretinal gene therapy in treating Bbs10-/- mice at moderately advanced disease.
Methods: The mouse Bbs10 gene was cloned into a shuttle plasmid containing a CMV promoter. This plasmid was then packaged into an AAV2/Anc80 viral vector (L65 variant). Bbs10-/- (KO) mice were aged to 3 months-old (MO) and were subretinally injected with 2μL of virus at either 8×10^8 vg/μL or 4×10^9 vg/ μL (ddPCR) for total doses of either 1.6×10^9 vg/eye or 8×10^9 vg/eye. Optical coherence tomography was used to analyze the outer nuclear layer (ONL) containing photoreceptor nuclei; this was completed at 1-, 3-, 5-, 7-, and 9-months post-injection (MPI). Data quantification was performed using ImageJ software. An electroretinogram (ERG) was used to assess the electrical function of the retina and was completed at the same timepoints as OCT. The swim assay which evaluates functional vision was completed at two timepoints: between 3-5 MPI and between 8-11 MPI.
Results: At 3MO, the average ONL thickness in KO mice was roughly 50% of that of age-matched heterozygous (HET) or wild-type mice (WT). At 3-MPI the average ONL of treated KO eyes were thicker than contralateral untreated eyes ($p = 0.0109$ and $p = 0.0062$). In assessing rod-specific function, there was a dose-dependent improvement with mice treated with 8×10^9 vg/eye the dose displaying the greatest amplitudes ($p = 0.037$). B-wave amplitudes of combined rod and cone function also exhibited a dose-dependent improvement with 1.6×10^9 vg/eye treated mice showing the highest amplitudes ($p = 0.045$). In light-adapted tests, b-waves amplitudes in treated eyes were

comparable to untreated eyes. The average light swim times at 8-11 MPI were 38.1s (untreated), 25.9s (1.6×10^9 vg/eye), and 24.5s (8×10^9 vg/eye). The average dark swim times at 8-11 MPI were 39.4s (untreated), 29.8s (1.6×10^9 vg/eye), and 26.9s (8×10^9 vg/eye).

Conclusions: Comparison of ONL thickness in 3MO KO mice vs HET or WT mice confirmed mid-stage retinal degeneration at the starting point for treatment. At 3MPI, ONL was significantly preserved in treated eyes compared to untreated eyes. This cell preservation finding was supported by increased rod-specific function as well as combined rod and cone function in both treated groups compared to untreated eyes in dark testing at 3MPI. In the light, there was no improvement in treated groups vs untreated groups, suggesting that the number of cones rescued is insufficient to stimulate electrical responses in full-field ERG. Treated mice in both the light swim and dark swim showed a trend toward dose-dependent improvements in swim time. This data provides proof of concept that treating mid-stage disease with subretinal gene therapy may rescue rod function greater than cone function and offers hope that treating patients at typical ages of presentation may offer some benefit.

Poster #11

Diurnal Variation Contributes to Cyst Severity in a Mouse Model of X-Linked Juvenile Retinoschisis

Stanley, Sarah[1]; Rankin, Tyler[1,2]; Laird, Joseph[1]; Thompson, Jacob[1]; Salma, Hassan[1]; Baker, Sheila[3]; Hsu, Ying[1]; Drack, Arlene[1]

UNIVERSITY OF IOWA DEPARTMENTS OF [1]OPHTHALMOLOGY AND VISUAL SCIENCES; [2]BIOCHEMISTRY AND MOLECULAR BIOLOGY; [3]INTERDISCIPLINARY GRADUATE PROGRAM IN GENETICS

Purpose: X-linked juvenile retinoschisis (XLRS) is an inherited vitreoretinal disorder caused by a hemizygous mutation in the gene RS1, located on the X chromosome. RS1 encodes for the protein retinoschisin, a protein thought to be essential for adhesion and organization of retinal layers. Patients diagnosed with XLRS, typically young males, present with schisis and formation of fluid filled cysts in the inner nuclear layer (INL) of the retina. This results in decreased central vision due to macular dystrophy and an increased risk of retinal detachments and/or vitreous hemorrhage. Previous clinical observations show a variation in cyst area and schisis levels depending on the time of day. Our lab was interested in exploring this diurnal phenomenon in an Rs1 knockout mouse model (Rs1-KO) to observe if these findings could be replicated.
Methods: Rs1-KO mice were aged between 3 and 4 months old. Optical coherence tomography (OCT) with central B scan imaging was used to analyze anatomical retinal architecture and measure cyst area at 2 timepoints, one morning and one evening time. In experiment 1, OCT acquisition was done in the light during the mouse's light cycle and in the dark during the mouse's dark cycle. Anesthesia with a ketamine/xylazine mixture followed by a reversal agent were administered to mice in experiments. Mice were dark adapted overnight, followed by timepoint 1 OCT at 5 AM in dark conditions. Second timepoint OCT were performed 84 hours later at 5 PM in light conditions. To determine if lighting during OCT acquisition influenced results, experiment 2 was performed with all OCT acquisition in light conditions, regardless of the mouse's dark or light cycle. All other conditions were the same from experiment 1. Cyst area was measured via Photoshop for all experiments.
Results: OCT: Cyst area was significantly larger at the morning timepoints than the evening timepoints in experiment 1, with a 70.30% reduction in cyst area. Experiment 2 followed a similar trend with a 65.60% reduction in cyst area from timepoint 1 to timepoint 2.
Conclusions: A mouse model of XLRS shows larger cyst areas in the morning than the evening timepoints, suggestive of a diurnal effect. This finding is not dependent on light conditions during OCT acquisition. These results illustrate the importance of consistency with the timing of OCT acquisition for XLRS in both the laboratory and potentially clinical setting.

Poster #13

Mechanisms of Kv2.1/Kv8.2 associated vision loss

Baker, Sheila[1]; Inamdar, Shivangi[1]; Berkowitz, Bruce[2]; Laird, Joesph[1]
[1]Department of Biochemistry and Molecular Biology, University of Iowa; [2]Wayne State University

Purpose: Patients with Cone Dystrophy with Supernormal Rod Response (CDSRR) experience a childhood-onset decline in cone mediated vision followed by macular degeneration. About half of the patients also develop night blindness. CDSRR is caused by mutations in KCNV2, which encodes the obligatory Kv8.2 subunit of the rod and cone specific heteromeric Kv2.1/Kv8.2 voltage-gated potassium channel. Kv2.1/Kv8.2 participates in setting the resting dark current and filtering light responses. We have initiated studies to uncover the cause of photoreceptor degeneration.
Methods: We developed two complimentary mouse models to study this disease. ERG, OCT, and metabolomics were used.
Results: Kv8.2 KO have the same electrical abnormalities as CDSRR, delayed or decreased rod responses in response to dim or brighter light respectively and decreased cone responses. In Kv8.2 KO there is slow rod degeneration and no loss of cones. Conefull: Kv8.2 KO retina has the same decrease in cone-driven electrical responses as Kv8.2 KO and there is accelerated cone degeneration compared to the all-cone retina with intact Kv8.2. Metabolomics was used to identify potential contributors to degeneration triggered by loss of Kv8.2. The two pathways most significantly altered in Kv8.2 KO retina were homocysteine degradation and taurine metabolism. High levels of homocysteine in neurons are associated with oxidative stress and inflammation. Taurine is an antioxidant and osmolyte abundant in photoreceptors that is essential for neuronal viability. Using a non-invasive biomarker we found light-triggered osmotic swelling of the subretinal space was lost in Kv8.2 KO.
Conclusions: Together our Kv8.2 KO and Conefull: Kv8.2 KO mice allow us to model the different regions of the human retina affected by CDSRR. Rods appear to protect cones from degeneration, which could explain why the degeneration in CDSRR patients is limited to the macula. Further investigation of osmotic dysregulation, oxidative stress and inflammatory signaling in this disease is warranted to achieve the larger goal of identifying targets for drug development to slow retinal degeneration.

Poster #15

Analyzing Palpebral Fissure Width to Brow Fat Span Ratio in Female Celebrities

Ram, Sibani[1]; Shriver, Erin2; Parikh, Rupin[2]
[1]Carver College of Medicine, University of Iowa, [2]Department of Ophthalmology and Visual Sciences, University of Iowa

Purpose: The periocular region has long been an area of analysis in the assessment of human female aesthetic appeal. Specifically, the increase of the tarsal platform show (iTPS) to brow fat span (iBFS) ratio has been associated with post-operative aesthetic appeal among women undergoing blepharoplasties. In addition, in several Asian-American countries, the presence of a large palpebral fissure width (PFW) has also been associated with greater aesthetic appeal. sing these past studies as a starting point while combining the eastern and western periocular aesthetic metrics, this project examines the palpebral fissure width (PFW) to brow fat span(BFS) ratio among the top ten actresses on Ranker.com's most recent listing of "Actresses with Most Captivating Eyes." In analyzing the PFW: BFS ratio in these ten actresses, a pattern emerges that a PFW:BFS ratio above 0.90 may be a characteristic associated with "captivating" eyes.
Methods: The top ten Caucasian actresses listed on Ranker.com's list of "The Most Captivating Celebrity Eyes (Women)" were analyzed using ImageJ software. The analysis included a measurement (in Pixels) of the PFW and the BFS. The PFW measurement is the space between the medial and lateral canthus of the eye. The BFS measurement utilized in this project is the distance from the eyelid crease to the top of the brow. These two numbers were then compared as a ratio of PFW:BFS after a conversion to Pixels to mm was completed.
Results: 8/10 of the celebrities had a PFW:BFS greater than or equal to 0.90.
Conclusions: In combining the widely accepted Western periocular aesthetic for increased brow fat span and the Eastern one of increased palpebral fissure width, it was determined that a PFW:BFS ratio at or above 0.90 predominated among the top ten actresses listed in Ranker.com's most captivating celebrities. It is crucial to note that all subjects analyzed Caucasian celebrities. As a result, the results of this study may not be generalizable to all women (or all actresses) across the world. However, this ratio does provide a metric that may point to a proportionality that is correlated with attractiveness and captivation in Caucasian celebrities.

Poster #17

Orbital Echographic Assessment of Extraocular Muscle Size Changes associated with Teprotumumab

Chun, Lindsay[1]; Fleecs, Julia[1]; Warner, Laura[1]; Pham, Chau[1]; Carter, Keith[1]; Shriver, Erin M.[1]
[1]Department of Ophthalmology and Visual Sciences, University of Iowa

Purpose: Changes in EOM size were assessed via orbital echography for adult patients who completed all 8 infusions of teprotumumab for TED. Clinical characteristics were also assessed.
Methods: This retrospective study evaluated the EOM diameters of adult patients with TED who underwent orbital echography before, during, and after their teprotumumab treatment period by a single experienced echographer. The total EOM size was obtained by summing the diameters of the four measured rectus muscles of each eye. The study eye was designated to the more proptotic eye prior to treatment.
Results: Twenty-six patients (20 female, 6 male, mean age 54.02 years) were included in this study. At a mean 9.98 weeks prior to the first teprotumumab initiation, the mean total EOM diameters were 26.69 mm and 26.71 mm in the study and non-study eyes, respectively. At the initial post-treatment follow-up (mean 6.64 weeks after the 8th infusion), there was a significant decrease in the mean total EOM diameters of the study eyes (24.60 mm, 7.83% decrease, p=0.001) and non-study eyes (24.57 mm, 8.03% decrease, p=0.003). At the final post-treatment follow-up (mean 41.12 weeks after the 8th infusion), there was a significant decrease in the mean total EOM diameters of the study eyes (24.69 mm, 7.49% decrease, p=0.01) and non-study eyes (24.68 mm, 7.61% decrease, p=0.02). There were also significant improvements in the mean values of CAS, motility, Hertel, and subjective diplopia seen after the completion of the 8th infusion (p±0.05 for all 4 measures).
Conclusions: To date, this study is the largest ultrasound-based investigation in the assessment of EOM size changes from teprotumumab for TED. These findings demonstrate that orbital echography is an effective imaging modality for TED that can show significant reduction in extraocular muscle diameter after teprotumumab.

Poster #19

Effects of ocular blast pressure injury on neurons and glia in the visual thalamus

Van Hook, Matthew[1]; Smith, Jennie[1]
[1]Truhlsen Eye Institute, Department of Ophthalmology and Visual Sciences, University of Nebraska Medical Center

Purpose: Ocular blast injuries can trigger traumatic optic neuropathy from shearing forces exerted on optic nerve, which is comprised of the axons of retinal output neurons called retinal ganglion cells (RGCs). RGCs project to visual centers of the brain such as the dorsolateral geniculate nucleus (dLGN), which is responsible for transmitting signals to the cortex for conscious vision. The goal of this study was to determine how an ocular pressure injury affects the cytoarchitecture of the dLGN.
Methods: A bilateral closed-globe ocular overpressure injury was induced in adult C57Bl6/J mice of both sexes with a 21 psi burst of nitrogen delivered to the eye from a modified Helios Gene Gun. Six weeks post-injury, scotopic electroretinogram (ERG) responses were recorded and brain tissue was harvested and sectioned for immunofluorescence studies. Immunofluorescence labeling and 2-photon microscopy was used to quantify blast-induced changes in neuron density (NeuN), microglia density and morphology (Iba1), optic tract astrocytes (GFAP), and RGC axon terminals (vGlut2).
Results: There was no detectable effect of ocular blast on ERG a- or b-wave amplitudes, although the b/a-wave ratio was higher at dim intensities in the blast-injured eyes. In the optic tract dorsal to the dLGN, there was no detectable difference in GFAP staining intensity. There was a modest reduction in NeuN cell density in blast injured dLGN sections, pointing to a loss of dLGN neurons. The density of vGlut2-labeled RGC axon terminals was also slightly lower in dLGN sections from blast-injured mice. There was no significant difference in the number of dLGN microglia between blast-injured and control tissue, although microglia showed signs of polarization, as indicated by reduced total branch length per microglial cell. Moreover, both microglia branch length and the number of process endpoints per microglia cell significantly correlated with vGlut2 density, pointing to a relationship between microglia polarization and RGC synapse loss.
Conclusions: Ocular blast injury leads to subtle changes in the cytoarchitecture of the dLGN, including changes in microglia morphology, loss of dLGN neurons, and loss of RGC axon terminals. Disruption to retinal projection targets might contribute to vision impairment following ocular blast trauma.

Poster #21

A Clinical Trial in a Dish to Investigate Neuroprotection and Remyelination in Optic Neuropathies

Hayes, Michael[1,2]; Valdes Michel, Maria F.[1,2]; Kardon, Randy H.[1,2]; Gramlich, Oliver W.[1,2]

[1]Department of Ophthalmology and Visual Sciences, University of Iowa; [2]VA Center for the Prevention and Treatment of Visual Loss, Iowa City

Purpose: Demyelinating diseases, such as multiple sclerosis (MS), result in neuronal loss effecting the visual and motor-sensory systems. Immunosuppression, the mainstay of treatment, effectively decreases disease severity and relapse. However, immunosuppressants are associated with severe adverse events, provide minimal neuroprotection, and fail to promote remyelination. Induced pluripotent stem cell (iPSC) derived organoids, three-dimensional organ-like cultures that recapitulate human tissue in vitro, are an ideal model to characterize candidate compounds. However, organoid based approaches are costly, time-consuming, typically underpowered, and suffer from batch-to-batch variability. Thus, we are developing real-time assays to increase efficiency, standardize cohorts, and improve data quality; ultimately creating an efficient, scalable, and customizable platform to investigate neuroprotection and remyelination in human tissue- our Clinical Trial in a Dish.

Methods: Naïve health donor and transgenic RGC specific Brn3-GFP reporter human iPSC lines were used within this study. iPSCs were maintained in mTeSR+ media. Pluripotency was confirmed via immunofluorescence and differentiated into retinal and oligodendrocyte rich cortical (oligocortical) organoids. Organoid differentiation was followed via brightfield microscopy. RGC development and function was visualized via Brn3-GFP expression and whole-organoid electrophysiology. The presence of oligodendrocytes, neurons, and astrocytes in oligocortical organoids was confirmed via MBP, Nestin, and GFAP immunofluorescent staining. Confocal microscopy was further paired with live cell compatible clearing methods to visualize Brn3-GFP expression and internal structures with vital stains. Cell survival was analyzed via live/dead staining following MS-like demyelinating and inflammatory injury. A custom organoid electrophysiology apparatus was used to measure RGC electrical activity in response to light.

Results: Compared to brightfield and epifluorescence microscopy, confocal imaging of cleared living organoids provides better resolution of individual cells and internal structures. Brn3-GFP expression demonstrated the presence of RGCs as early as day 21 of retinal differentiation and revealed axon-like projections within neuroretinal tissue and across non-neuroretinal organoid sections at later time points. At day 56, these cells were shown to respond to full-field light, in an intensity-dependent manner and

RGC characteristic prolonged hyperpolarization. Neurons, astrocytes, and oligodendrocytes were observed within oligocortical cultures. Of note, oligodendrocytes were observed to have linear MBP+ structures suggestive of axonal myelination. MS-like inflammatory and demyelinating injury resulted in cell death, observed at single cell resolution in live organoids.

Conclusions: We have successfully differentiated human iPSCs into oligodendrocyte rich cortical organoids and retinal organoids containing functional RGCs. We have developed live-cell compatible fluorescent staining and clearing methods to facilitate visualization of internal structures and improve image quality without the need to sacrifice, fix, and process organoids. These advances, paired with our innovative live-cell reporter strategy, will facilitate the establishment of an efficient, scalable, and customizable platform to investigate neuroprotection and remyelination in human tissue-our Clinical Trial in a Dish.

Poster #23

Beyond Retinal Thickness: Textural Changes Precede Retinal Thinning in Acute Non-Arteritic Ischemic Optic Neuropathy (NAION)

Wang, Jui-Kai[1,2]; Woods, Brian[3]; Chen, Zhi[4]; Zhang, Honghai[4]; Szanto, David[5]; Sonka, Milan[4]; Garvin, Mona[2,6]; Linton, Edward [1,2]; Kupersmith, Mark[5]; Kardon, Randy [1,2]

[1]Department of Ophthalmology and Visual Sciences, University of Iowa; [2]VA Center for the Prevention and Treatment of Visual Loss, Iowa City; [3]Irish Clinical Academic Training Programme; [4]Iowa Institute for Biomedical Imaging, University of Iowa; [5]Department of Neurology, Icahn School of Medicine at Mt. Sinai, NY; [6]Department of Electrical and Computer Engineering, University of Iowa

Purpose: Optical coherence tomography (OCT) can visualize retinal nerve fiber layer (RNFL) and ganglion cell inner plexiform layer (GCIPL) loss in non-arteritic anterior ischemic optic neuropathy (NAION). While thickness maps are used to evaluate structural changes associated with edema and atrophy, RNFL en-face images may provide additional textural information (e.g., showing regionally lower intensity but hyper-reflectivity on vessels) that precedes changes in thickness, as an earlier sign of neuron damage when intervention may improve outcome.
Methods: OCT scans of 142 NAION patients in the acute stage (≤15 days from onset of vision loss) and 50 days after symptom onset were analyzed. RNFL and GCIPL en-face images and thickness maps were generated from OCT volumes after layer segmentation using a novel deep-learning approach. Mean RNFL and GCIPL en-face pixel intensity and thickness were computed.
Results: RNFL en-face pixel intensity decreased significantly in the acute NAION stage compared to the fellow eye, but not RNFL or GCIPL thickness. Both RNFL en-face intensity and RNFL/GCIPL thickness all decreased after 50 days from symptom onset; fellow unaffected eyes showed no significant change over time.
Conclusions: Pixel intensity maps of the inner retinal layers reveal early NAION damage in the acute stage. Decreased RNFL en-face pixel intensity suggests that early textural change has potential to predict future structural thinning of the inner retina. Texture-based biomarkers based on en-face images show promise for detecting pre-thinning damage in NAION versus thickness measures alone.

Poster #25

Endothelin-1 overexpression elicits cellular elastinopathy and reactive astrocytosis in rat optic nerve head astrocytes

Betancourt-Szymanowska, Candace[1]; Rao, Vidhya R.[1]; Kaja, Simon[1]
[1]Department of Ophthalmology, Loyola University Chicago

Purpose: Glaucoma is a progressive optic neuropathy characterized by optic nerve head (ONH) remodeling, damage to the optic nerve, and retinal ganglion cell loss. Optic nerve head astrocytes (ONHA) are the primary glial cell in the ONH. Noxious stimuli trigger reactive astrocytosis (RA), a morphological and structural remodeling associated with increased expression of glial fibrillary acidic protein (GFAP), enhanced proliferation and migration, reduced stellation, changes in actin cytoskeleton, and altered secretion of extracellular matrix proteins. RA is an early pathophysiological process contributing to glaucoma, which is thought to underlie the characteristic ONH remodeling. Glaucoma patients have been shown to have increased levels of the peptide endothelin-1 (ET-1) in aqueous humor and serum in vessels surrounding the eye. This study's objective was to investigate the putative role of endothelin-1 (ET-1) on RA, specifically elastin pathway gene expression, in ONHA.
Methods: We generated primary rat ONHA cultures overexpressing human V5-tagged endothelin 1 (EDN1-ONHA) and a V5-tagged FLAG (Control ONHA) by lentiviral transduction. Overexpression of EDN1 was confirmed by qPCR and ELISA after puromycin selection. We characterized differences in elastin pathway expression, and endogenous endothelin receptors A (Ednra) and B (Ednrb) because of EDN1 overexpression via qPCR and immunoblotting. We characterized differences in cell morphology via immunofluorescence microscopy, and assessed differences in cell proliferation rates by MTT proliferations assays and cytometry.
Results: EDN1-ONHA expressed increased levels of ET-1, as quantified by ELISA (1.3 pg/mL in EDN1-ONHA vs. ±0.4 pg/mL in control ONHA). EDN1-ONHA exhibited a less-differentiated morphology, significantly enhanced proliferation rates, and increased GFAP expression, suggestive of RA. Gene expression of proteins involved in elastin signaling, specifically fibulin 2 (Fbln2), fibulin 5 (Fbln5), lysyl oxidase like-1 (Loxl1) and elastin (Eln) was reduced by 24% (p±0.05), 63% (p±0.001), 29% (p±0.05) and 63% (p±0.01), respectively. Decreased expression of Loxl1 and Eln was confirmed by immunoblotting, showing a 48% reduction in Loxl1 (p±0.05) and a 62% reduction in Eln (p±0.05). Gene expression for endothelin receptor A (Ednra) was similar between EDN1- and control ONHA (p=0.84). In contrast, endothelin receptor B (Ednrb) was upregulated 5-fold (p±0.05).
Conclusions: Overexpression of human ET-1 in rat ONHA elicits induction of characteristic features of RA, supporting a pathological role of ET-1 in glaucomatous

optic nerve head remodeling. Notably, ET-1 induced a phenotype of elastinopathy, with similar decreases in Loxl1 and Eln levels as previously observed following exposure to mechanical strain. Loss of elastin during RA may cause impaired elastic fiber formation and stabilization and contribute to decreased biomechanical compliance of the optic nerve head during glaucoma. Upregulation of Ednrb mRNA levels is consistent with the role of Ednrb as a “clearance receptor" for ET-1. Ongoing research is elucidating the signaling pathways mediating these transcriptional changes in the elastin pathway and EDNRB activation.

Poster #27

Nicotinamide Induces Cobblestone Morphology in ARPE-19 Cells but Not in Primary RPE Cells: A Comparative Study

Luscan, Joshua[1]; Waite, Matthew[1]; Schubert, Brynn [1]; Markand, Shanu[1]
[1]A.T. Still University-Kirksville, College of Osteopathic Medicine

Purpose: The ARPE-19 cell line is widely used as an alternative to primary RPE cells in many eye research studies because of its relative convenience and consistency and the scarcity of donor eyes. One limitation of this alternative is that ARPE-19 cells do not exhibit cobblestone morphology, resulting in an undifferentiated state and cytoskeletal disorganization. This study tests the effects of nicotinamide, a member of the vitamin B family, on the differentiation of the ARPE-19 cell line with different FBS concentrations to see if the growth rate of the cells plays a role in cobblestone morphology. This study also tests the effects of nicotinamide on primary RPE cells derived from donor eyes to see if there is any change in shape and for a baseline comparison with the ARPE-19 cells.
Methods: ARPE-19 cells were grown in DMEM F-12 media supplemented with varying FBS concentrations and 1% Penicillin/Streptomycin. Cells were grown in a 6-well culture plate and maintained at 37˚C in a humidified incubator with 5% CO2. The media were replaced with fresh media three times per week. The varying FBS concentrations comprised three groups: 0.1%, 0.2%, and 0.5%. ARPE-19 cells were grown in DMEM F-12 treated with ten mM nicotinamide, with the same varying FBS and Penicillin/Streptomycin concentrations, for five days. The primary RPE cells had a similar experimental setup and were maintained for approximately one month.
Results: After five days of treatment, the cells in the control group appeared elongated and spread out. The cells in the FBS treatment group were more tightly aligned with each other, exhibiting cobblestone morphology. Interestingly, after treatment for a longer period, no morphological change in shape was observed between the control and treatment groups in primary RPE cells.
Conclusions: ARPE-19 cells treated with ten mM of nicotinamide exhibited cobblestone morphology compared to the control group. This observation is most likely due to nicotinamide's role in cellular metabolism. As for the primary RPE cells, nicotinamide did not affect cell differentiation, size, or health. Future studies will validate the origins of primary RPE cells and prolong the treatment time in ARPE-19 cells to see if increased duration results in further morphological changes and sustainability.

Poster #29

Evaluation of the Choroidal Sprouting Assay as an Ex Vivo Model of Microvascular Angiogenesis

Shravah, Varun[1]; Rao, Vidhya R.[1]; Kaja, Simon[1]
[1]Stritch School of Medicine, Loyola University Chicago

Purpose: The choroidal sprouting assay is an ex vivo model commonly used to study microvascular angiogenesis. This model can help assess pathways involved in the proliferation of choroidal microvessels and screen compounds as potential treatments for ocular conditions, such as neovascular age-related macular degeneration (AMD) and proliferative diabetic retinopathy (PDR). However, one major limitation of this assay is that it has not been used to study choroidal vascular tissue in organisms besides mice and rats. The goal of this project was to evaluate the ability of rodent, rabbit, and human choroids to undergo microvascular angiogenesis and to optimize the experimental conditions to support testing of anti-angiogenic drugs.
Methods: Segments of mouse, rabbit, or human choroid were isolated from whole eyes and seeded in basement membrane extract (Matrigel®) on a 24-well plate and incubated for 48 hours at 37ºC/95% humidity/5% CO2. Variations in growth medium, Matrigel® composition and age of the donor were evaluated. Choroidal explants were imaged every 1-3 days using an imaging plate reader (Cytation 5, Agilent) with automated multifocal and multiview acquisition. Sprouting was quantified using ImageJ 1.46r.
Results: Choroid sprouting was observed in CSC and EBM-Plus growth media, but not with DMEM medium. Choroids from C57BL/6J mice at P2 sprouted most robustly and showed faster sprouting than tissue from older animals (P60 or P144). Growth factor reduced Matrigel® still resulted in significant non-stimulated choroidal sprouting. Rabbit choroids from 7.5 month old New Zealand White rabbit showed robust sprouting in CSC and EBM-Plus media. Choroidal tissue from a 75-year-old male human donor showed evidence of sprouting in CSC and EBM-Plus media, although sprouting was less robust and slower than that observed with mouse or rabbit tissue.
Conclusions: The choroid sprouting assay can be successfully reproduced across different species. To our knowledge, this is the first report of ex vivo choroidal sprouting from rabbit or human donor tissue. Choroidal sprouting had an inverse relationship with the age of the donor, with aged mouse tissue sprouting significantly less than neonatal tissue. CSC and EBM-Plus media provide better growth conditions for sprouting compared to DMEM media. Ongoing research is aimed at standardization of the ex vivo choroidal sprouting assay to incorporate possible species-specific responses and allow for better translatability to subsequent in vivo testing in the drug discovery process.

Poster #31

A deep learning model for evaluating fundus image quality in the UK Biobank dataset

Hsu, Ying [1]; Michaelson, Jacob[2]
UNIVERSITY OF IOWA, DEPARTMENTS OF [1]OPHTHALMOLOGY AND VISUAL SCIENCES; [2]PSYCHIATRY

Purpose: Features in retinal fundus images can be used to predict eye diseases or diseases of the central nervous system using convolutional neural networks. The UK Biobank dataset includes 67,321 fundus images of subjects in the United Kingdom along with visual acuity, ophthalmic and family history, biometrics, overall medical history, and mental health surveys. However, to prepare large fundus datasets for training a deep learning model, image quality control must be performed and images with low quality and notable artifacts should be excluded. We describe a deep learning model that automatically generates fundus image quality assessment comparable to quality assessments performed by an expert human grader.
Methods: A 16-layer convolutional neural network was designed using RStudio to perform automatic image quality assessment using the University of Iowa Interactive Data Analytics Service, a high-performance computing resource. The train, validation, and test datasets consist of 100, 100, and 50 fundus images, respectively. Images in the training set were randomly sampled from sites including Middlesborough, Hounslow, Croydon, Birmingham, Swansea, Wrexham, the validation set sampled from Bristol, Barts, Nottingham, Sheffield, Liverpool, and the test set from the remaining 11 sites. Image quality was graded by a human grader. The quality scores range between 0 to 5, with 5 being an image of excellent quality, and 0 being an image with no visible features due to poor quality. Quality scores by the human grader were divided by 5 to yield scores between 0 and 1. The model outputs quality scores between 0 and 1, and the mean absolute error between human assessment and model prediction is reported.
Results: The deep learning model generates automatic image quality assessments comparable to a human grader with a mean error of 0.15 per image. The model generates quality scores for 50 test images in 1.147 seconds using 32 CPU cores, 512 G of RAM, and an NVIDIA L4 GPU.
Conclusions: This convolutional neural network can automatically assess fundus image quality and outputs quality scores comparable to a human grader. This deep learning model can be used to perform quality control for large fundus image datasets such as the UK Biobank.

Poster #33

N-acetylcysteine (NAC) suppresses retinal defects in Drosophila models of SNRNP200-associated Retinitis Pigmentosa

Wallrath, Lori[1]; Mayer, Sara [1]; Christensen, Quinton [1]; McCoy-Munger, Hailey[1]; Nathanial P., Mohar[1]; Drack, Arlene V.[1]

UNIVERSITY OF IOWA, DEPARTMENTS OF [1]BIOCHEMISTRY AND MOLECULAR BIOLOGY; [2]OPHTHALMOLOGY AND VISUAL SCIENCES

Purpose: Retinitis Pigmentosa (RP) is a genetic disorder characterized by progressive retinal degeneration that can lead to complete blindness. RP affects 1 in 4,000 individuals worldwide and can be either syndromic or non-syndromic. Early stages of RP involve death of rod photoreceptors via apoptosis, causing loss of peripheral and night vision. This is followed by the death of cone photoreceptors, leading to loss of central vision. Mutations in over 300 genes cause RP. Many of these genes encode retina-specific proteins; however, several encode globally expressed proteins, such as pre-mRNA splicing factors. For example, mutations in the SNRNP200 gene encoding, a core pre-mRNA splicing factor, cause non-syndromic RP by mechanisms that are not well understood. Currently, there are no treatments for individuals with SNRNP200-associated RP.

Methods: An approach to understanding the molecular pathogenesis of rare diseases is to generate the human disease-causing mutations in model organisms where molecular and genetic tools are available. As such, we used CRISPR gene editing to generate Drosophila models in which RP-causing mutations were introduced into the highly conserved Drosophila orthologue Snrnp200. In addition, we used the Gal4/UAS system to express an RNAi against Snrnp200 to knock-down levels in the retina. To test for effects of an antioxidant on mutant phenotypes, flies were allowed to ingest media containing N-acetylcysteine (NAC) throughout development.

Results: Depletion of Snrnp200 caused a “rough eye" phenotype, which is indicative of cell death. To further investigate this, we immunostained and found that knock-down of Snrnp200 increased apoptosis in larval eye discs relative to controls. Heterozygous mutant alleles of Snrnp200 modeled after human RP mutations caused defective patterning of the photoreceptors, abnormal mitochondria, and abnormal electroretinography, relative to wild-type flies. Consistent with these finding, the mutants show altered regulation of redox genes, suggesting a loss of redox homeostasis. To determine if altered redox was contributing to the pathology, the antioxidant N-acetylcysteine (NAC) was fed to the flies. This treatment partially rescued the photoreceptor defects, suggesting that oxidative stress contributes to retinal defects and

that NAC is a potential avenue for therapy for individuals with SNRNP200-associated RP.

Conclusions: Depletion of Snrnp200 increases apoptosis in the developing Drosophila eye. Snrnp200 mutant alleles cause dominant defects of the photoreceptors, yet no additional overt abnormal phenotypes, similar to tissue-restricted human disease symptoms. Photoreceptors in the mutants exhibit abnormal patterning, enlarged and increased numbers of mitochondria, and changes in gene expression, relative to controls. These abnormalities are accompanied by abnormal electroretinograms. Collectively, our findings demonstrate the utility of a Drosophila model for studies of SNRNP200-RP. The mitochondrial defects and changes in redox gene expression support loss of redox homeostasis. Partial rescue of the phenotypes by NAC suggests a possible treatment for individuals with SNRNP200-RP.

Poster #2

A Clinical Challenge: Delayed Diagnosis of Autoimmune Polyglandular Syndrome Type II in a Patient with Thyroid Eye Disease

Hartness, Emma[1]; Garza Reyes, Arnulfo [2]; Parikh,Rupin N.[2]; Lepa, Nicholas J.[3]; Rahimi, Leili[4]; Shriver, Erin M.[2]

[1]University of Iowa Roy J. and Lucille A. Carver College of Medicine; [2]Department of Ophthalmology and Visual Sciences, University of Iowa; [3]Department of Emergency Medicine, Mercy Medical Center, Cedar Rapids, Iowa; [4]Division of Endocrinology, Diabetes, Metabolism, and Nutrition, Department of Medicine, Mayo Clinic, Rochester, Minnesota

Purpose: Autoimmune polyglandular syndrome type 2 (APS-II) is a rare diagnosis comprising 2 or more autoimmune endocrinopathies in one patient. Reports discussing thyroid eye disease (TED) associated with APS-II are lacking in the ophthalmic literature. The case presented here is unusual in that while up to 50 percent of patients who develop autoimmune primary adrenal insufficiency develop APS-II, only 1 percent of patients with autoimmune thyroid disease develop autoimmune primary adrenal insufficiency.

Methods: A 35-year-old woman with a history of TED presented to her oculoplastics follow-up with a history of new-onset symptoms characteristic of exacerbated TED. She decided to treat her symptoms with NSAIDs and to schedule close follow-up, however over subsequent months developed worsening headaches, severe gastrointestinal discomfort, labile blood sugars, and hyponatremia. She underwent 8 visits to the emergency department with supportive care until she developed persistent hyponatremia and hyperkalemia and received a targeted endocrine workup.

Results: The patient demonstrated low 8 a.m. serum cortisol and an inadequate response to an adrenocorticotropic hormone (ACTH) stimulation test. She was diagnosed with APS-II and began a daily steroid regimen. On follow-up in the oculoplastics clinic, she had significant improvement in her symptoms and stable signs of TED.

Conclusions: Ophthalmologists should remain vigilant regarding patients with headaches or orbital pain which are new in onset or changed in character. Those who encounter patients with autoimmune primary adrenal insufficiency, autoimmune thyroid disease, or type I diabetes should advocate for close monitoring by a PCP or referral to an endocrinologist for evaluation and screening to facilitate an accurate diagnosis and treatment.

Poster #4

Characterization of immune populations in cervical lymph nodes in Ad5 viral vector model of glaucoma

Jones, Merri-Grace[1,2]; Xian, Shuyu[1,2]; Kuehn, Markus, H.[1,2]
[1]Department of Ophthalmology and Visual Sciences, University of Iowa; [2]VA Center for the Prevention and Treatment of Visual Loss, Iowa City

Purpose: Glaucoma is a leading cause of blindness world-wide and significantly reduces quality of life. Elevated intraocular pressure (IOP) is a significant risk factor for primary open angle glaucoma (POAG). However, lowering IOP reduces but does not inhibit the progression of retinal ganglion cell (RGC) death in POAG. This suggests that other factors besides IOP contribute to RGC death. We previously published that lymphocytes contribute to RGC loss in animal models of glaucoma. However, the immune response following the induction of glaucoma has not been characterized. In this study, we wanted to determine if there is a correlation between upregulation of IOP and changes in immune populations cervical lymph nodes following induction of glaucoma.
Methods: Mouse eyes received an injection of the adenoviral vector Ad5RSVmyocillin^{Y437H} (University of Iowa Viral Vector Core) to induce glaucoma. Newborn (P2-P4) B6 mice received a subcutaneous injection of Ad5myoc (3×10^3 PFU) to induce tolerance to the vector and prevent ocular inflammation. At eight- to 12- weeks of age, 9×10^7 PFU virus was delivered into the anterior chamber of the eye by micropump injection. The development of IOP was monitored using a rebound tonometer (Tonolab). IOP measurements were taken weekly between 9 am and 1 pm by a researcher blinded to the animals' treatment condition. Mice with elevated IOP and control animals at various time points (14- or 28-days post-injection) were euthanized and cervical lymph nodes were harvested. Spectral flow cytometry was used to quantify and phenotype immune cell populations in the cervical lymph nodes at these time points.
Results: Between 14- or 28-days post-injection, we observed an increase in number of various immune subsets in the cervical lymph nodes of Ad5-injected mice. Specifically, in the T cell compartment we noted an increase in the number of CD8 T cells (P=0.0448) and Tregs (P=0.0462). We also observed an expansion of MHC II+ non-lymphocytes (P= 0.0001) and conventional dendritic cells (P= 0.0004) in Ad5-injected mice overtime. However, when we assessed the correlation between median IOP and numbers of immune subsets, we observed no significant correlation.
Conclusions: In summary, these data suggest that the immune response following Ad5 viral vector induced glaucoma occurs within 4 weeks post-injection and is prior to RGC

damage. Treatments that target this immune dysregulation may be a promising avenue to inhibit the progression of RGC death in glaucoma patients.

Poster #6

Implementation of a Mediterranean Diet Exacerbates Visual and Motor-Sensory Impairment in an Animal Model of Multiple Sclerosis

Pablo, Julian[1]; Anders, Jeffrey[2,3]; Elwood, Benjamin[2,3]; Kardon, Randy[2,3]; Gramlich, Oliver W.[2,3]; Elwood, Benjamin[2,3]

[1]University of Iowa Carver College of Medicine; [2]Department of Ophthalmology and Visual Sciences, University of Iowa; [3]VA Center for the Prevention and Treatment of Visual Loss, Iowa City

Purpose: Multiple Sclerosis (MS) is a chronic neurodegenerative autoimmune disease that leads to demyelination of the central nervous system (CNS). This can lead to increasing paralysis and visual dysfunction. Around 2.5 million people worldwide currently live with MS. Approximately 20-45% patients experience optic neuritis (ON) as the initial presenting symptom. Various studies report ameliorative effects when implementing a Mediterranean diet in regard to motor-sensory and visual symptoms of MS-ON patients. Therefore, the aim of this study was to determine the neuroprotective effects of the Mediterranean diet (MD) on an experimental autoimmune encephalomyelitis (EAE) mouse model of MS-ON.
Methods: EAE was induced in 80 female C57BL/6J by immunization with MOG33-55. Complete Freund's Adjuvant, and pertussis toxin. Cohorts of EAE mice (n=20/group) were assigned to stay on standard rodent chow (EAE group), or to start the Mediterranean diet either 2 weeks prior EAE induction (pre[conditioning] MD group), at the time of EAE induction (pro[phylactic] MD group), or at the onset of initial presentation of symptoms (late MD group). A separate naïve group was made up of 20 unaffected mice. Mice were scored everyday based on motor-sensory symptoms from 0 (normal) to 5 (death) during the totality of the 6-week study. Visual acuity was tested via their optokinetic responses on a weekly basis. Retinal ganglion cell (RGC) complex layer thickness was measured at baseline, day 21, and day 42 using optical coherence tomography (OCT). Retinas, optic nerves, brain, and spinal cord were all harvested for analysis. Data was analyzed using one- and two- way ANOVA followed by post hoc tests.
Results: Preconditioned and Prophylactic animals showed significantly worse EAE scores when compared to untreated EAE animals (Area under curve EAE score: EAE: 53.31.5x, EAE + pre MD: 64.8±2.5, p=0.0013; EAE + pro MD: 71±2, p±0.0001; EAE + late MD: 57.6±2.5). Animals from the prophylactic group also had lower visual acuity when compared to untreated EAE animals (EAE: 0.267±0.1 cycles/degree vs. EAE + pro MD: 0.166±0.13 cycles/degree, p=0.01). The preconditioned group (0.213±0.12 cycles/degree) and late group (0.244±0.13 cycles/degree) both showed no significant

difference in visual acuity scores compared to the EAE group. A significant decrease in RGC complex layer thickness was observed in all EAE groups compared to naïve controls (67.7±2.3 µm, p±0.001), but also between the prophylactic group and the EAE group (EAE: 64.7±3.2 µm vs. EAE pro MD: 62.6±3.8 µm, p=0.029).
Conclusions: This study found that the implementation of a Mediterranean diet worsened motor-sensory impairment and exacerbated the visual system's decline in structure and function in all EAE -ON animals. Moreover, EAE mice in the prophylactic MD group experienced significantly worse outcomes in visual acuity and RGC complex thickness as measured by OCT. Subsequent histopathologic examinations will provide a more in-depth insight in the pathobiology of ON which are likely to confirm the ophthalmic outcome measurements. However, the results indicate that the Mediterranean diet may have a negative effect on MS patients and further studies are needed to determine the optimal diet for neuroprotection and rehabilitation of the CNS after demyelination.

Poster #8

Enhanced attention in rats following blast-induced traumatic brain injury

Harper, Matt[1,2]; Navarro, Victor[1,2]; Boehme, Nickolas[1,2]; Wasserman, Edward[1,2]
[1]Department of Ophthalmology and Visual Sciences, University of Iowa; [2]VA Center for the Prevention and Treatment of Visual Loss, Iowa City

Purpose: To evaluate visuo-cognitive sequelae following blast-induced traumatic brain injury in a rat model.
Methods: Rats were randomly assigned to one of four groups depending on the intensity/quantity of a blast received in a blast chamber: sham (no blast), low intensity (22 psi), medium intensity (26 psi), or three medium intensity blasts (26 psi □ 3). After recovery, all subjects were given visual discrimination tasks of increasing complexity, until mastery. After behavioral training, visual function was assessed via spectral-domain optical coherence tomography and pattern electroretinogram, and the extent of retinal damage was quantified via immunohistochemistry of retinal ganglion cells.
Results: None of the measures assessing visual function revealed significant differences as a function of blast intensity/quantity. Behavioral training did not disclose short-term effects of blast in general motivation or the development of anticipatory responding. No differences in general learning ability and the number of perseverative errors were observed. However, behavioral training found effects of blast in attentional function; relative to controls, subjects that received blasts were faster in learning to attend to informative (over non-informative) cues in the most difficult visual discrimination task.
Conclusions: Blast exposure in rats resulted in increased attention following blast, with no appreciable deficits in visual function. These results are contrary to what is often reported for human clinical populations; as such, more research bridging methodological differences is necessary.

Poster #10

Comparison of trabeculectomy approach on surgically-induced astigmatism in the treatment of open-angle glaucoma

Al-Kaylani, Hend[1]; Dang, Hansen[1]; Mays, Christian[2]; Boese, Erin[2]; Sears, Nathaniel[2]; Pouw, Andrew[2]
University of Iowa [1]Carver College of Medicine; [2]Department of Ophthalmology and Visual Sciences

Purpose: Open-angle glaucoma is a chronic disease that is multifactorial in origin. The primary impact on patient quality of life is progressive, irreversible vision loss. Trabeculectomy is the gold-standard surgical approach to slow progression of the disease when medical or laser therapies fail to control intraocular pressure. While trabeculectomy is highly effective at controlling glaucoma, it typically introduces astigmatism. This was a retrospective observational study comparing the average change in surgically-induced astigmatism (SIA) across four different trabeculectomy methods (limbal-based, fornix-based, and with or without partial-thickness limbal corneal incision) at the University of Iowa Hospitals and Clinics.
Methods: A retrospective chart review was conducted for all patients that underwent trabeculectomy for open-angle glaucoma at the University of Iowa Hospitals and Clinics between August 2020 - August 2023. Exclusion criteria included combined phacotrabeculectomy, retinal or corneal comorbidities, concurrent ocular surgeries, and patients under 18 years of age.
The pre-operative wearing refraction and post-operative manifest refraction noted within 2 years of the procedure were compared to evaluate for SIA change. One-tailed ANOVA and Tukey's Honest Significant Difference test were performed to compare statistically significant differences between surgical approaches on induced astigmatism. This study was approved by the Institutional Review Board for Human Subjects Research at the University of Iowa.
Results: A total of n=87 patients were included in the final analysis (out of 381 initial observations). The mean age of the sample was 73.1 ± 11.7 years. 59.8% of our sample was female. 25 procedures had a limbus-based approach by Surgeon 1, 23 fornix-based by Surgeon 1, 18 fornix-based by Surgeon 2, and 21 fornix-based by Surgeon 3. While the mean change in cylinder difference between surgical methods ranged from 0.913 diopters (standard deviation ± 0.617) to +1.35 diopters (SD ± 1.47), there was no significant difference in cylinder change among the four surgical approaches (p=0.409). No two methods differed significantly from each other in induced cylinder change.

Conclusions: This study was an opportunity to compare four different trabeculectomy approaches on surgically-induced astigmatism in a single institution. We found that, within our sample, all four surgical approaches studied increased the magnitude of astigmatism post-operatively. There was no significant difference in induced postoperative cylinder change between surgical approaches in our sample population. While there is excellent research comparing limbus- and fornix-based trabeculectomy on failure rate, change in pressure, and number of medications required post-operatively, there is limited work comparing change in astigmatism. Thus, this study adds insight on the effect of trabeculectomy approach on SIA. Future work comparing SIA of these approaches can guide clinicians in predicting refractive changes, individualizing treatment decisions with patients, and advising patients on what to expect after surgery.

Poster #12

Astrocyte Origin and Developmental Stage Influences Ability to Guide Retinal Ganglion Cell Outgrowth

Thallapureddy, Khyathi[1]; Abukunna, Fatima[1]; Kador, Karl[1]
[1]Department of Ophthalmology, University of Missouri-Kansas City

Purpose: During development, retinal ganglion cells (RGCs) axons are guided to the optic nerve head by both ECM bound and secreted factors from the cells of the optic nerve head (ONH). Interestingly, this guidance is not observed in either retinal organoids or when RGCs are transplanted into adult animals. To study this guidance, cells were isolated from the ONH of early developmental and adult rats as well from glia in developing retina outside of the ONH and from the developing cortex, and cultured with RGCs to determine the ability to direct RGC neurite growth.
Methods: Primary cells were isolated from postnatal day-2 (P2) and adult Sprague Dawley rat ONH, and glia isolated from P2 retina. These cells as well as cell lines for type 1 and type 2 cortical astrocytes from P2 mice were expanded in vitro before being positioned at the center of radial scaffold. RGCs from early postnatal rats were seeded on the scaffold surrounding the different glia and cultured for 3 days after which the cells were fixed and stained. RGCs were analyzed for growth towards the positioned glia, away from the cells or in both directions for each glial population. P2 ONH cells were then purified via immunopanning into different sub-populations to determine which cells were responsible for increased guidance.
Results: P2 ONH cells positioned in matrigel at the scaffold center polarized up to 60% of RGCs while no other cell population was able to increase guidance above control samples of matrigel alone. Astrocytes from this cell population were observed to increase guidance while also increasing the health of seeded RGCs.
Conclusions: Optic nerve head astrocytes isolated from the developing retina increased retinal ganglion cell growth polarization towards the center of our biodegradable scaffolds. The origin including the location from which they are isolated as well as the developmental stage of the astrocytes plays a role in their ability to polarize RGCs growth. The study provides an in vitro model for understanding the interaction between RGCs and astrocytes and the potential therapeutic targets of astrocytes in glaucoma.

Poster #14

Dynamic Retinal Blood Flow Analysis: Heartbeat-Correlated Artery and Vein Identification in Laser Speckle Flowgraphy

Takahashi, Noriyoshi[1]; Wang, Jui-Kai[1,2]; Linton, Edward F.[1,2]; Ahmad, Noor-Us-Sabah[1,2]; Garvin, Mona K.[2,3]; Kardon, Randy H.[1,2]

[1]Department of Ophthalmology and Visual Sciences, University of Iowa; [2]VA Center for the Prevention and Treatment of Visual Loss, Iowa City; [3]Department of Electrical and Computer Engineering, University of Iowa

Purpose: Laser speckle flowgraphy (LSFG) is a non-invasive imaging technique employed to evaluate retinal and choroidal blood flow. Traditionally, analyzing the blood flow of retinal arteries and veins in LSFG involves manually identifying regions of interest on the recorded video and average blood flow map, a process that can be both tedious and subjective. This study introduces a novel, automated approach for the identification of retinal arteries and veins in LSFG by utilizing temporal blood flow variations across heartbeats as multi-channel inputs to nnU-Net.

Methods: The dataset comprised 30 training and 10 test LSFG data. First, Expert1 determined the arteries and veins of retinal vessels based on the fundus photograph, and then Expert2 filled in the retinal vessel area of the LSFG composite blood flow map based on the results to create a reference trace label image. The input to the deep learning model was a video of blood flow fluctuations during one heartbeat, divided into 10 frames and assigned to 10 channels. The existing GAN-based method (ASBFN) and our proposed model were trained on the same data. Comparisons were also made with the standard analysis software used when analyzing LSFG.

Results: The proposed method achieved Dice coefficients of 0.78 ± 0.06 for arteries and 0.79 ± 0.06 for veins, significantly outperforming an existing method with coefficients of 0.61 ± 0.16 and 0.63 ± 0.07, respectively. Moreover, the proposed method demonstrated significantly higher recall and F1 scores in identifying artery and vein segments surrounding the optic nerve, compared to the standard analysis software (paired t-test p-values ± 0.01). In terms of counting vessel segments and quantifying blood flow, the proposed method showed no significant differences from manual tracing (paired t-test p-values $\gg$ 0.05).

Conclusions: This study represents an important advancement toward fully automated retinal blood flow analysis, reducing manual intervention and enhancing diagnostic accuracy and efficiency in the assessment of diseases affecting blood flow.

Poster #16

Improvements in Documentation and Billing of Ophthalmology Diagnostic Testing

Yang, Lydia[1]; Kuhn, Molly[1]; Pouw, Andrew[1]
[1]University of Iowa Carver College of Medicine

Purpose: Electronic Health Records (EHRs) have revolutionized clinical practice but largely remain under-optimized. Inconsistent practices in clinical documentation of ophthalmic test results create billing inefficiencies and potential medicolegal liabilities for clinicians if tests are billed without formal documentation of clinical interpretation. We implemented a simple logic tool in Epic (Epic Systems, Verona, WI) that redirected clinicians to documentation of tests if any were done during clinic visits, before chart closure.
Methods: We implemented a “Close Encounter Warning" using Epic CER rules to redirect clinicians to the Imaging and Procedures section of the Epic chart for documentation of test interpretation. Chart closure (signing the Clinic Visit encounter “closed") was allowed if the logic rules did not encounter any unsigned testing. The CER logic rules underwent many revisions to account for the complexities of the workflow in the Ophthalmology department and to account for the mixture of fellows, residents, and staff that worked in each patient’s chart. The initial iteration was implemented on 10/24/21, and the final iteration was implemented on 2/8/22. We compared the number of closed charts with unresulted diagnostic tests in the UIHC Ophthalmology department between January 2021 and November 2023 to measure the impact of the close-encounter rule.
Results: The number of closed charts with unresulted diagnostic tests in the UIHC Ophthalmology department was compared before and after the implementation of the imaging Close Encounter Warning. Prior to the implementation of the close-encounter rule, the average number of charts closed with unresulted diagnostic images was 924.1 per month. Following implementation, it decreased to 9.2 per month, a 100-fold decrease.
Conclusions: The implementation of the Close Encounter Warning effectively reduced the number of unresulted diagnostic tests in the UIHC Ophthalmology department. This change in the EHR was able to significantly reduce inefficiencies in billing coding and reduced potential medicolegal liability for our clinicians for incorrect billing practices.

Poster #18

Particulate Matter Elicits Ocular Toxicity Through Multiple Distinct Cellular and Biochemical Pathways

Kaja, Simon[1]; Ghosh, Anita K.[2]; Pasupuleti, Bhuvanachandra[3]; Rao, Vidhya R.[1]; Sana, Iqbal[1]

[1]Ophthalmology, Loyola University Chicago; [2]Program in Biochemistry and Molecular Biology, Loyola University; [3]Program in Molecular Pharmacology and Therapeutics, Loyola University

Purpose: Toxic particulate matter (PM) is generated by the incomplete combustion of organic and inorganic material. PM is present in urban air pollution derived from vehicular and industrial exhausts, agricultural burning and wildfire smoke, and household biomass combustion. There is extensive clinical evidence that PM is linked to several conditions, including ocular surface disease (OSD). Knowledge of the multifactorial pathophysiology underlying PM-induced OSD is lacking. The objective of this study was to investigate the cellular and biochemical mechanisms of PM-associated ocular toxicity.

Methods: Standard Reference Material PM4 (SRM® 2786) and PM10 (SRM® 2787) were obtained from the National Institute of Standards and Technology. Assays were performed in primary and immortalized human (pHCEC, HCE-T) and rabbit (SIRC) corneal epithelial cells, human LAD2 mast cells, and primary human neutrophils. Effects of PM on corneal epithelial cell viability and proliferation were assessed by MTT uptake and LDH release assays; generation of cytosolic and mitochondrial ROS were quantified using DCFDA and MitoSOX sensors, respectively. Effects on cellular motility were determined by scratch assay. Cytokine release was quantified by multiplex immunobead assay. Mast cell degranulation was determined by ELISA using prostaglandin D2 and histamine. Neutrophil activation was quantified by flow cytometry, while adhesion of neutrophils to HCE-Ts was determined using calcein-labeled neutrophils and plate-reader based quantification of fluorescence. Data were analyzed and graphed in Prism software.

Results: Upon exposure to PM4 and PM10 (1 – 300 μg/mL), corneal epithelial cells showed a dose-dependent loss of cell viability, elevated levels of cytosolic and mitochondrial oxidative stress, increases in the release of pro-inflammatory cytokines, and impaired motility. Neutrophil expression of CD11b (alphaMbeta2 integrin) and CD16 (FcγRIIIa) increased significantly after exposure to PM4 and PM10 as assessed by flow cytometry, suggestive of neutrophil activation. PM4 resulted in increased adhesion of primary neutrophils to corneal epithelial cells. In LAD2 mast cells, exposure to PM4, and to a lesser extent PM10, resulted in secretion of increased levels of PGD2 and histamine, suggestive of mast cell degranulation.

Conclusions: "These results identify multiple cellular and biochemical pathways that together contribute to the ocular toxicity elicited by PM. These include direct toxic effects on corneal epithelial cells, increased levels of oxidative stress, an allergic response and neutrophil activation. Notably, PM4 caused greater toxicity compared with PM10, confirming our prior in vivo studies in rabbits. Utilizing well-characterized and standardized PM in validated experimental assays amenable to high throughput screening will allow for the identification of the drug candidates that target some or all of these pathophysiological pathways. Similarly, these assays can be used to test effects of other environmental pollutants and toxins.
Using a targeted medicine approach, our ongoing studies are testing and developing biologics and small molecules for their ability to mitigate PM-associated ocular toxicity.

Poster #20
<a name="Pasupuleti"

Novel approaches to evaluating drug candidates to mitigate antibody-drug conjugate chemotherapy associated corneal toxicity

Pasupuleti, Bhuvanachandra[1]; Iqbal, Sana [1]; Tidei, Joseph J.[2]; Kaja, Simon[3];
[1]Program in Molecular Pharmacology and Therapeutics, Loyola University; [2]Program in Cellular and Molecular Physiology, Loyola University; [3]Ophthalmology, Loyola University Chicago

Purpose: Antibody-drug conjugates (ADC) have emerged as promising chemotherapeutic agents for multiple types of cancer. However, various ADC are associated with significant off-target corneal toxicity attributed to the non-specific corneal uptake of ADC by macropinocytosis. The overall goal of this study was to develop an unbiased high-content imaging approach allowing the quantification of macropinocytosis-mediated cellular uptake. ADC-mediated cytotoxicity and cell cycle arrest were quantified to determine the feasibility of repurposing imipramine, an FDA-approved macropinocytosis inhibitor, as a therapeutic strategy to combat ADC-mediated ocular toxicity.
Methods: To quantify MPC-mediated cellular uptake, human corneal epithelial cells (HCE-T) were seeded in black/clear bottom 96 well plates. Cells were exposed to a dose range of imipramine (0.1-10 μM), 5-[N-ethyl-N-isopropyl] amiloride (EIPA; 25 - 100 μM) or vehicle for 24 h, followed by Dextran-Texas RedTM (10,000 KDa; 0.5 mg/mL) for an additional 3.5 h. Plates were imaged using a Cytation 5 multimodal plate reader. Fluorescence intensity and immunopositive area were quantified using a custom FiJi macro. To analyze cell cycle arrest, HCE-T cells were seeded in T25 flasks and pre-incubated with either vehicle, drug treatments (ADC, belantamab mofodotin: 0.15 – 45 μM, colchicine: 500 nM, imipramine: 0.5-10μM or EIPA: 25–100 μM) for 24 h. Cells are trypsinized, fixed in 70% ethanol, and labeled with propidium iodide (50 μg/mL) in the presence of RNAase (1mg/ml). Cell cycle was analyzed by flow cytometry using a FortessaTM flow cytometer. Data were graphed and analyzed using Prism 10 software.
Results: Dextran Texas Red fluorescence intensity and immunopositive area increased significantly after exposure, suggesting potent MPC activity in HCE-T cells. EIPA significantly reduced fluorescence intensity and immunopositive area by ~80%. Imipramine resulted in a significant reduction of fluorescence intensity, suggestive of MPC inhibitory activity, but the level of reduction was ~20%.
Belantmab mafodotin exerted dose-dependent cytotoxicity, consistent with previous reports. Concentrations as low as 0.15 nM resulted in notable cell cycle arrest. However, the effect was too small to reach sufficient power to determine any effect of imipramine. Higher doses of belantamab mafodotin (4.5 nM) resulted in a statistically

significant reduction of the G1/G2 ratio of the cell cycle. However, imipramine did not protect against belantamab mafodotin mediated cell cycle arrest."
Conclusions: "Our data support the use of plate reader-based imaging as a feasible approach for assessing MPC-mediated uptake into corneal epithelial cells. The method can be readily expanded to high-throughput screening approaches to identify drug candidates with activity against MPC in corneal epithelial cells.
Although imipramine reduced MPC-mediated uptake, none of the concentrations tested were able to reduce MPC by >20%. Based on cytotoxicity and cell cycle studies, this inhibitory effect was insufficient to protect HCE-T cells from belantamab mafodotin-induced toxicity.
Ongoing studies are investigating the feasibility of using higher doses of imipramine and are evaluating other FDA-approved drug for their ability to inhibit MPC in corneal epithelial cells.

Poster #22

Changes in Spinal Cord Gene Expression correlates with Motor-Sensory Impairment in an EAE Model of Optic Neuritis

Valdes Michel, Maria F.[1,2]; Anders, Jeffrey J.[1,2]; Capper, Erin N.[1,2]; Chimenti, Michael[3]; Kardon, Randy H.[1,2]; Gramlich, Oliver W.[1,2]

[1]Department of Ophthalmology and Visual Sciences, University of Iowa; [2]VA Center for the Prevention and Treatment of Visual Loss, Iowa City; [3]Iowa Institute of Human Genetics, University of Iowa, Iowa City, IA

Purpose: Inflammation of the optic nerve, termed optic neuritis (ON), is a common manifestation of autoimmune demyelinating disorders that affect the visual system. These include myelin oligodendrocyte glycoprotein associated disease (MOGAD), neuromyelitis optica spectrum disorder (NMOSD), and multiple sclerosis (MS). Experimental autoimmune encephalomyelitis (EAE) is one of the most common animal models of MS and MOGAD. EAE animals experience bilateral ON and spinal cord pathology. The purpose of this study is to: 1) identify relevant pathways and biological processes within the spinal cord of EAE animals compared to the SHAM controls and 2) correlate the visual phenotype of the EAE model with mobility impairment and gene expression changes in the spinal cord.
Methods: For EAE, 11 six-week-old wildtype C57BL/6 female mice were immunized with MOG35–55, emulsified in Complete Freund's adjuvant (CFA). For SHAM control, 6 six-week-old female mice were injected with CFA emulsified in ddH2O. All mice were then injected with 2 doses of pertussis toxin. Clinical progression was monitored using a 5-point EAE scoring scheme. Visual acuity was assessed by optokinetic response (OKR) and RGC complex thickness was measured by OCT. At the conclusion of the 6-week study spinal cords from each group were collected and RNA isolated for transcriptomic analysis via bulk RNA sequencing. Differential expression (DE) analysis between specified groups was performed using DESeq2 in Partek Flow. Genes with adjusted P value (± 0.05) and log2 fold change (≥2.0 or ≤ –2.0) were labelled as differentially expressed. Furthermore, enrichment analysis for affected biological processes and functional pathways was performed in iPathwayGuide.
Results: EAE animals show significant motor-sensory impairment compared to SHAM controls, including decreased visual acuity (EAE:0.194±0.014 c/d vs. SHAM: 0.3912±.014 c/d; p±0.0001) and RGC complex thinning versus SHAM controls (EAE:60.1±1.45μm vs. SHAM:67.9±1.45μm; p±0.0001). We observed DE in 1,198 out of 20,192 genes. Gene ontologic analysis identified enrichment of complement activation (p= 3.263e-8) and negative regulation of cytokine production (p =1.000e-24) in EAE versus SHAM animals. Pathway analysis found that genes related to the cytokine-cytokine receptor interaction (p =1.998e-24) and Epstein-Barr virus infection

(p=5.422e-24) were highly upregulated in the EAE mice when compared to their controls. Additionally, we observed significant correlation between the mobility impairment (AUC) and top DE genes. Notable but non-significant correlations were observed between visual function and AUC (p=0.0705), H2-M2 (p=0.0789), Fcgr2d (0.0776) and Fcgr4 (p=0.0931).
Conclusions: Our data demonstrate a significant correlation between motor-sensory impairment and gene expression changes in the spinal cord. While the visual phenotype does not show a significant correlation, we observed a trend towards negative correlation. Although a correlation between these parameters was anticipated, it is plausible that spinal cord gene expression changes are distinct from alterations in the visual system.

Poster #24

Knockout of component C3 in complement cascade decreases photoreceptor function without concomitant cell loss

Smith, Jennie C[1]; McCool, Shaylah[1]; Thompson, Jennifer[2]; Fan, Shan[1]; Van Hook, Matthew J.[1]

[1]Truhlsen Eye Institute, Department of Ophthalmology and Visual Sciences, University of Nebraska Medical Center; [2]Department of Pharmacology and Experimental Neuroscience, University of Nebraska Medical Center

Purpose: Complement component C3 plays an essential role in the classical complement cascade, a large group of plasma proteins and regulatory cell surface proteins activated by pathogens, pathogen-antibody complexes, or spontaneously that lead to a chain reaction of products that help or “complement" the immune system via destruction of pathogens and removal of dead cells and foreign material. The goal of this study was to determine how the loss of complement C3 impacts retinal function and structure.
Methods: C3 knockout mice on a C57Bl6 background (Jax: #003641) were used at 8-12 weeks (young) and 7-9 months (aged) of age. Controls were age-matched C57Bl6J mice. In-vivo electroretinogram (ERG) was used to determine function of outer retina (photoreceptors and bipolar cells) and inner retina (amacrine cells). Scotopic ERGs of both young and aged and photopic ERGs of aged cohorts were accomplished. Optical Coherence Tomography (OCT) of young and aged mice used light waves to take cross-section pictures of the retina. We averaged 2 independent measurements of retinal layers ONL+ELM (outer) and RNFL+GCL+IPL (inner). Images of retinal cross-sections from young knock-out and control mice were used for initial comparison of ONL (photoreceptors) thickness. Fixed eye-cups were sliced in 25 um-thick sections, then DAPI-stained to count nuclei. Triplicate counts were taken near the optic nerve head (ONH) and sextuplicate taken distal from ONH. Nested ANOVA and t-tests were used for statistics.
Results: Young knock-outs showed a reduced ERG a-wave and b-wave compared with controls, but no significant difference in the sum of Oscillatory Potentials (OPs). Aged mice showed no significant variance in a-wave or b-wave except when segregated by sex, aged female knock-outs had a reduced a-wave compared to controls. Young knock-outs had a reduced photopic b-wave compared to controls. There was no significant difference in a-wave, and no statistical differences when segregated by sex. Using OCT, we found that knock-outs showed no significant change in inner or outer retina thickness as measured at 500 nm or 1000 nm from the optic nerve head. Counts

of DAPI-stained photoreceptor somata in the outer nuclear layer also indicated no significant difference between KO and controls, consistent with OCT findings. **Conclusions**: This mouse model indicates the absence of C3 from the complement system can lead to degradation of rod and cone pathway function, as measured by ERG analysis, although without a detectable loss of cells, as seen in OCT imaging and DAPI-stained retinal sections. Future work is needed to determine the mechanisms of reduced ERG amplitudes.

Poster #26
<a name="McCool"

Microglia Polarization in the Visual Thalamus of an AD Mouse Model

McCool, Shaylah[1]; Smith, Jennie[1]; Van Hook, Matthew[1,2]
[1]Truhlsen Eye Institute, Department of Ophthalmology and Visual Sciences, University of Nebraska Medical Center; [2] Department of Cellular and Integrative Physiology, University of Nebraska Medical Center

Purpose: Understanding the effect of amyloid-beta (Aβ) plaques, a defining pathology of Alzheimer's disease (AD), on visual brain regions is critical as deficits in vision are a major barrier to high quality of life for AD-affected individuals. The retina synapses with thalamocortical (TC) neurons in the dorsal lateral geniculate nucleus (dLGN), a thalamic region that processes visual information from the retina and transports it to higher cortical regions. As the dLGN is a retinorecipient brain region, this makes it an excellent target to study visual deficits in AD. The 5xFAD mouse of amyloidosis will be used to study the involvement of microglia in the immune defense of the dLGN in AD. We hypothesize that microglia polarization, due to Aβ plaque formation, is associated with loss of retinal ganglion cell (RGC) axon terminals and TC neurons in the dLGN. Examining the activity of immune cells in the dLGN may indicate how the visual system is responding to formation of Aβ plaques during AD
Methods: For this work, we used male and female 6-, 9-, and 12-month 5xFAD mice. This mouse develops Aβ plaques throughout the brain, including the dLGN, in an age-dependent manner. This model allows us to study the effects of Aβ separate from that of tau tangles. Age-matched C57BL/6J mice were used as controls as the 5xFAD mice are bred on a C57BL/6J background. The primary methods employed for this project are immunohistochemistry, 2-photon imaging, and skeleton analysis of microglia. We stained for vesicular glutamate transporter 2 (vGlut2) to mark RGC axon terminals present in the dLGN while staining of neuronal nuclei (NeuN) was used to count TC neurons in the dLGN. Ionized calcium binding adapter protein 1 (Iba1), a cytoskeletal protein, was used to tag microglia. Finally, we performed a skeleton analysis to analyze microglia polarization by examining two morphological features – branch length and endpoints per cell.
Results: At 9 months, we see a loss of vGlut2 density in the 5xFAD dLGN. However, by 12 months, the C57 mice have lost equivalent amounts of vGlut2. Counting neurons in the dLGN using NeuN staining, we found a decrease in neuronal density at 6 and 12 months in the dLGN of the 5xFAD mice. 2-photon imaging of Iba1-stained brain sections revealed significantly lower branch length per microglia and endpoints per microglia in the 5xFAD mice at all three time points compared to controls. This increase

in microglial polarization is associated with decreased vGlut2 density at 9 months as well as fewer NeuN-positive cells at all timepoints.
Conclusions: Overall, we are seeing accelerated loss of vGlut2 in the 5xFAD mice. Interestingly, we also found decreased neuron density in the 6-month and 12-month 5xFAD mice, but not at 9 months. The skeleton analysis shows that the microglia in the 5xFAD mouse dLGN are significantly more polarized compared to age-matched controls. These more polarized microglia are also correlated with fewer RGC axon terminals in the 9mo 5xFAD dLGN as well as being correlated with fewer neurons at all timepoints in the 5xFAD mice. Future work for this research includes skeleton analysis of the suprachiasmatic nucleus (SCN), another retinorecipient brain region that reportedly does not develop Aβ plaques in this model.

Poster #28

A Longitudinal Analysis of a MOG Induced Mouse Model of Multiple Sclerosis-like Optic Neuritis

Anders, Jeffrey[1,2]; Elwood, Benjamin[1,2]; Kardon, Randy[1,2]; Gramlich, Oliver[1,2]
[1]Department of Ophthalmology and Visual Sciences, University of Iowa; [2]VA Center for the Prevention and Treatment of Visual Loss, Iowa City

Purpose: Optic neuritis (ON) is often an early sign of multiple sclerosis (MS) occurring in nearly 40% of newly diagnosed MS patients. The ratio of MS diagnosis is 3:1, female to male which lends to a large sex component of the disease. However, males tend to show more severe symptoms throughout the disease course. Additionally, an ON episode within these patients usually presents as a unilateral ON episode. This study aimed to create a 12-week longitudinal database of the Experimental Autoimmune Encephalomyelitis (EAE) model of MS like ON. This study will allow us to compare the structural and functional changes within the visual system.
Methods: EAE-related ON was induced in 65 male and female C57BL/6J mice by immunization with MOG33-55, Complete Freund's Adjuvant and pertussis toxin. Another 65 male and female served as naïve control. Five male and female EAE and control animals were euthanized from weeks 0-12. Clinical progression was monitored using a 5-point EAE scoring scheme. Visual acuity was assessed weekly while RGC complex thickness and retinal nerve fiber layer (RNFL) was measured using optical coherence tomography (OCT) for baseline and euthanasia date. Differences between control and EAE groups as well as sex differences and OD vs. OS were determined using a two-way ANOVA and Bonferroni's post-hoc test.
Results: EAE animals symptom onset starts around day 12 for both sexes. The peak of disease happens around day 21 for both sexes. EAE animals show significant decline in visual function starting at week 2 lasting until end of study when compared to control animals (EAE: 0.33 c/d vs CNTRL: 0.41 c/d; p±0.0001). EAE mice showed significant thickening of the retinal nerve fiber layer at week 2 (EAE: 31.65μm vs. CNTRL: 29.05μm; p=0.0004). This is followed by significant thinning in EAE mice starting week 10 which lasts to week 12 (EAE: 28.1μm vs. CNTRL: 29.85μm; p=0.0159). EAE mice showed a significant decrease in ganglion cell complex thickness that started at week 8 (EAE: 67.29μm vs. CNTRL: 73.18μm; p±0.0001). These structural changes do not vary between sexes. All visual acuity, motor sensory score, RNFL, and ganglion cell complex thickness are not significant between sexes. There were no significant differences between OD and OS eyes in RNFL and ganglion cell complex thickness in the EAE group.
Conclusions: This MOG induced EAE model represents one of the most used models to study MS. This study showed that like in acute episodes of ON in MS patients, this

model also shows a swelling within the RNFL layer. Additionally, the model also shows significant thinning of both the RNFL and ganglion cell complex after the acute phase as seen in MS patients. However, no model is perfect when recapitulating a disease. For instance, this study shows decreased visual acuity that does not recover which represents a more MOGAD-like phenotype, not MS. Additionally, this model shows a bilateral ON whereas MS usually presents as unilateral. In conclusion, this study represents an important longitudinal data set that can be used to study both MS and MOGAD phenotypes relating to structural and functional changes over time.

Poster #30

Histopathologic Evaluation of Human Optic Nerves in Multiple Sclerosis Patients

Capper, Erin[1]; Elwood, Benjamin[2,3]; Kardon, Randy[2,3]; Gramlich, Oliver W.[2,3]
[1]University of Iowa Carver College of Medicine; [2]Department of Ophthalmology and Visual Sciences, University of Iowa; [3]VA Center for the Prevention and Treatment of Visual Loss, Iowa City

Purpose: There is a current gap in literature describing the histopathologic characteristics of MS optic nerves at the axonal level. The purpose of our study was to examine optic nerves in MS and healthy controls in order to quantify axon density as well as characterize optic nerve pathology findings.
Methods: Optic nerves were fixed in 2% glutaraldehyde 2% paraformaldehyde from the eyes of 5 healthy and 7 MS patients. The optic nerves were cut longitudinally into quarters. Each quarter was prepared using a TEM embedding protocol. Semi-thin 500 nm sections were cut using an Ultramicrotome. The samples were transferred to slides and stained with PPD. Optic nerve sections were imaged broadly at 20x and then in four areas at 100x magnification (periphery, mid-periphery, mid-central, and central) using the Zeiss Axioscope. Axon counts were determined for each image in a semi-automatic manner using ImageJ plugin AxoNet. Data were analyzed using an unpaired t-test to determine if a difference existed between the axon density of control and MS optic nerves. A two-way ANOVA was used to determine if a difference existed between axon density at each of the four areas. Lastly, two representative optic nerve images, one control and one MS, were selected for descriptive histopathological analysis.
Results: Optic nerves from the MS cohort showed significantly decreased axon density compared to the control cohort (Mean axon density: MS: 799.1 axons/field, Control: 1267 axons/field, p=0.0360). Further analysis of axon density by region revealed significantly decreased axon density in the central area of MS optic nerves compared to controls (p=0.0053), but no significant difference was seen in the periphery, mid-periphery, or mid-central areas. Histopathological analysis revealed increased axonal loss and connective tissue formation in the MS optic nerve. The presence of demyelinated axons, myelin outfolding, and low-density myelin rings were observed in the MS sample only. Myelinoid bodies and detached myelin were seen in the MS and control optic nerve but were observed to a greater extent in the MS sample. Multilayered myelin was seen in both of the optic nerves and occurred to a similar degree in MS and healthy controls.
Conclusions: This study identified that there was significantly decreased axon density in the optic nerve of patients with MS when compared to healthy controls. Specifically, the axon loss was found to occur at the central region of the optic nerve. Additionally,

histopathological characterization of the MS optic nerve showed axon demyelination which was absent in the control optic nerve. Abnormal myelin pathology was observed in both the MS and healthy optic nerve analysis but occurred to a greater extent in the MS sample.

Poster #32

Microbead-Induced Ocular Hypertension and Optic Nerve Crush Reduce Ganglion Cell Complex Thickness in a Mouse Model of Alzheimer's Disease

Kragerud, Ben[1]; Thao, Mason[1]; Burkhalter, Rebecca[1]; Mathu, Virginia[2]; Oikawa, Kazuya[2]; Kiland, Julie A.[1]; McLellan, Gillian[1]

[1]Department of Surgical Sciences, UW-Madison School of Veterinary Medicine; [2]Department of Ophthalmology and Visual Sciences, University of Wisconsin-Madison

Purpose: Glaucoma is a neurodegenerative disease affecting the optic nerve , often caused by increased intraocular pressure (IOP). Damage to the optic nerve and ganglion cell complex (GCC, composed of the retinal nerve fiber, ganglion cell layer, and inner plexiform layer) results in vision loss. Optical Coherence Tomography (OCT) imaging enables measurement of retinal and GCC thickness in vivo. Alzheimer's disease (AD) is a neurodegenerative disease characterized by the build-up of β-amyloid (Aβ) plaques in the brain. Comorbidity between glaucoma and AD is common in human patients but the nature of this association remains unclear.

Methods: 5XFAD mice, which serve as a model for familial AD due to Aβ accumulation, and their wild-type (wt) littermates underwent optic nerve crush (or sham surgery) or microbead injection (or saline injection) or served as naïve controls. Optical coherence tomography (OCT) imaging was used to assess retinal thickness in mice in vivo (wt n=60; 5XFAD n=75.) Thickness of GCC was measured using Heidelberg Eye Explorer software, with manual correction as needed) in OCT images (Spectralis, Heidelberg) acquired under isoflurane anesthesia and mean values compared between groups by ANOVA and Tukey's multiple comparisons tests, with p±0.05 considered statistically significant.

Results: Optic nerve crush significantly reduced GCC thickness compared to their sham controls in all groups (5XFAD male p=0.0007, 5XFAD female p±0.0001, wt male p±0.0001, wt female p±0.0001). However, in microbead injected mice, mean GCC thickness was variable and reduction in GCC thickness in microbead injected mice was only statistically significant in female 5XFAD mice compared to naïve controls (p=0.002.) No Statistically significant difference in GCC thickness was identified between naïve 5XFAD and naïve wt mice at 9 months of age.

Conclusions: Our study demonstrates the utility of OCT in detecting the impact of both optic nerve crush and microbead-induced ocular hypertension on GCC thickness in mice in vivo. Our OCT findings suggest a differential susceptibility of female 5XFAD mice to GCC damage when subjected to high IOP, highlighting the importance of considering sex as a biological variable in AD and glaucoma research.

Poster #34

Laser Speckle Flowgraphy Reveals Widespread Reductions in Ocular Blood Flow in Non-exudative Age-related Macular Degeneration Independent of Ocular Perfusion Pressure

Linton, Edward F.[1,2]; Ahmad, Noor-us-Sabah[2]; Filister, Riley[3]Wang, Jui-Kai[1,2]; Sohn, Elliott[1]; Kardon, Randy[1,2]

[1]University of Iowa Department of Ophthalmology and Visual Sciences, Iowa City, IA; [2]VA Center for the Prevention and Treatment of Blindness, Iowa City; [3]Carver College of Medicine at the University of Iowa, Iowa City, IA

Purpose: Reduced perfusion in the choroid may contribute to accelerated age-related macular degeneration (AMD). Historical studies found reduced ophthalmic artery flow, and recent work highlights reduced capillary density at the leading edge of geographic atrophy. Laser speckle flowgraphy bridges these findings, providing quantitative information about blood flow velocity in the retina and choroid.
Methods: In our cross-sectional study, 24 subjects with non-exudative age-related macular degeneration in the early, intermediate or advanced stage underwent laser speckle flowgraphy and were compared to 21 age-matched control subjects. The main outcome measures were average choroidal blood flow and inner retinal blood flow in each group. Ocular perfusion pressure, eye characteristics, and AMD risk factors were compared between groups to determine whether differences in perfusion were explained by these factors.
Results: 39 eyes of 24 subjects with AMD and 41 eyes of 21 controls were included. Choroidal blood flow was reduced by 33% overall [mean blur rate 5.3<±0.3AU vs 7.9<±0.5AU, p=0.00005]. Inner retinal blood flow was also reduced [12.5<±0.6 vs 15.6<±0.5 AU, p=0.004]. Ocular perfusion pressure showed no significant difference between AMD and control groups (Normal 53<±6.7 mmHg vs AMD 50<±5.45mmHg; p=0.17 by t-test), indicating that elevated intraocular pressure or low blood pressure could not account for the reduced blood flow. In most cases, the area of lowest blood flow far exceeded the abnormal appearing AMD retinal area. Controlling for other subject and eye characteristics a 10%, 25% or 50% reduction in choroidal blood flow would be associated with a odds ratio having AMD of 2.27, 7.76, and 60.1, respectively (p=0.026).
Conclusions: Laser speckle flowgraphy showed lower choroidal and inner retinal blood flow in non-exudative AMD patients compared to age-matched controls, not explained by low blood pressure or elevated intraocular pressure. Areas of reduced blood flow greatly exceeded the territory of manifest pathology, emphasizing its role as a significant risk factor.