15th MIDWEST EYE RESEARCH SYMPOSIUM

Friday August 4, 2023

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 Dr. Tam

9:00-9:15	Connie Tam:	Epithelial cytokeratin 6a regulates secretory autophagy of cytokines to control corneal inflammation
9:15-9:30	Jade Enright:	The long noncoding RNA Gm11454 regulates retinal development
9:30-9:45	Bryce Shonka:	SOD2 in Fuchs Endothelial Corneal Dystrophy: Friend or Foe?
9:45-10:00	Noor-Us-Sabah Ahmad:	Laser speckle flowgraphy shows reduced ocular blood flow in dry age-related macular degeneration (AMD)

10-11 Poster Session I/Coffee

11-12 Platform Session II, Chaired by Dr. Vergara

11:00-11:15	Natalia Vergara:	Modeling Alzheimer's disease retinopathy with human iPSC-derived retinal organoids
11:15-11:30	Evelyn Craigen:	Cis-regulatory Element Activity in Temporal Regulation of Retinal Cell Fate
11:30-11:45	Sarah Brumley:	Reduced abundance of trabecular meshwork stem cells (TMSC) in human donor eyes with glaucoma
11:45-12:00	Jade Harkin:	Elucidating the influence of microglia on retinal ganglion cells in a human pluripotent stem cell model

12-1 Lunch

1-2 Platform Session III, Chaired by Dr. Baker

1:00-1:15	Sheila Baker:	KCNV2 Retinopathy: from eyeballs to atoms
1:15-1:30	Alanna Sullivan:	Novel nrf2 mutant zebrafish fail to regulate target genes and are susceptible to light-induced photoreceptor death
1:30-1:45	Jordan Mayberry:	Contribution of CD4 and CD8 cells to retinal ganglion cell loss in glaucoma
1:45-2:00	Zachary Heinzman:	Validation of the Iowa Head-Mounted Open-Source Perimeter

2-3 Poster Session II/Coffee

3-4 Keynote Address - Bärbel Rohrer, Ph.D
"Complement-Activation and Age-related Macular Degeneration: Lessons on Mechanisms and Treatments from Mouse Models"

4-4:30 Recognition of Outstanding Presentations

PLATFORM SESSION I

CHAIR: DR. CONNIE TAM

9-10 PM

Epithelial cytokeratin 6a regulates secretory autophagy of cytokines to control corneal inflammation

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PURPOSE: Epithelial cells form a crucial barrier against harmful microbes and inflammatory stimuli. Restraining inflammatory responses at the corneal barrier is necessary for avoiding sight-threatening tissue damage. Yet, epithelial cell-intrinsic mechanisms that dampen inflammation are largely unexplored. Keratin 6a (K6a) is a common type II cytokeratin highly expressed in corneal and other stratified epithelial cells. In a mouse model of sterile corneal inflammation, we observed disease exacerbation in K6a knockout mice. Here, we investigate a novel cell-intrinsic mechanism by which cytoplasmic K6a curbs corneal inflammation via regulation of secretory autophagy of cytokines.

METHODS: Human telomerase-immortalized corneal epithelial cells (hTCEpi) were either untreated or treated with inflammatory P. aeruginosa culture supernatant. Specific gene expression was knocked down by siRNA. Cytokines secreted to culture media were measured by ELISA. Protein expression was assessed by Western blotting of cell lysates. Subcellular localization of LC3-II, interleukin-8 (IL-8) and other autophagy-related proteins was detected by immunofluorescence confocal microscopy and transmission electron microscopy (TEM). To detect acidity of autophagosomes, cells were transduced with a baculovirus system producing an autophagy tandem sensor (RFP-eGFP-LC3B). Stable hTCEpi cell line expressing HA tagged-K6a was used to identify physical interactors of K6a by affinity purification followed by mass spectrometry.

RESULTS: Under both basal and inflammatory conditions, K6a-knockdown (K6a-KD) hTCEpi cells secreted higher levels of cytokines and chemokines (IL-1a, IL-6, IL-8, CXCL1, CCL20) as compared to wildtype cells. K6a-KD cells also had increased expression of LC3-II, a marker for autophagosomes, while the level of sequestosome-1 (SQSTM1/p62), a substrate for degradative autophagy, remained unchanged. In K6a-KD cells, the majority of LC3-II puncta were associated with non-acidified autophagosomes rather than acidified autolysosomes. Upon stimulation, IL-8 was found to co-localize with LC3-II. Mass spectrometric analysis of K6a immunoprecipitates identified Sec16a, a protein involved in secretory autophagy, as an interacting partner of K6a. Further experiments showed that knocking down key proteins involved in autophagosome formation (ATG5) and secretory autophagy process (GRASP55, Rab8a, Sec16a) abolished the augmentative effect of K6a-KD on cytokine and chemokine secretion.

CONCLUSIONS: These findings reveal a novel repressive role of K6a in secretory autophagy-mediated proinflammatory mediator secretion and provide new insights into cell-intrinsic mechanisms of inflammation control at epithelial barriers.

The long noncoding RNA Gm11454 regulates retinal development

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PURPOSE: In the developing retina, long noncoding RNAs (lncRNAs) are often expressed in a cell type specific and temporally dynamic manner. We propose that lncRNAs, known to regulate neurogenesis, influence the gene regulatory networks dictating cell cycle exit and cell fate specification during retinal development. In this study, we investigate the functions of the lncRNA Gm11454, which is enriched in late retinal progenitor cells (RPCs), in the regulation of retinal development.

METHODS: Gm11454 was overexpressed through in vivo and ex vivo electroporation of P0 mouse retinas. We generated a Gm11454 deletion mouse line using CRISPR/Cas9f. RPC cell cycle exit and cell fate were evaluated using immunohistochemistry. Knockout retinal structure and function were assessed using H&E staining and electroretinograms (ERGs). Gene expression changes to Notch pathway genes were detected by qPCR and further evaluated by electroporation of the CBF1 responsive reporter element (CBFRE) driving GFP expression.

RESULTS: P0 overexpression of Gm11454 promoted cell cycle exit and biased RPCs towards a photoreceptor cell fate. Conversely, Gm11454 knockout retinas have decreased cell cycle exit and decreased photoreceptors, as well as decreased inner layer neurons, compared to heterozygote. ERGs show impeded retinal function of Gm11454 knockout mice. Notch signaling was decreased in Gm11454 overexpressing cells with a corresponding increase in Gm11454 knockout retinas.

CONCLUSIONS: Taken together, our data suggest that the lncRNA Gm11454 functions in late RPCs to inhibit Notch signaling, thereby promoting cell cycle exit as well as photoreceptor cell fate, both of which require Notch downregulation. Gm11454 loss of function thus results in altered retinal development and function. Our results implicate lncRNAs as novel regulators of retinal development and retinal cell fate specification.

SOD2 in Fuchs Endothelial Corneal Dystrophy: Friend or Foe?

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PURPOSE: Oxidative damage, mitochondrial dysfunction, and UV sensitivity have been implicated in the pathogenesis of Fuch's endothelial corneal dystrophy (FECD). Manganese superoxide dismutase (MnSOD), a mitochondrial enzyme encoded by SOD2, is an important antioxidant for CEC function and is deficient in FECD. Ferroptosis is a mechanism of nonapoptotic oxidative cell death due to iron-mediated lipid peroxidation and has been demonstrated to be upregulated in FECD surgical tissue and cell culture models. This is of interest, as SOD2, in the presence of increased iron availability, forms FeSOD2, a prooxidant peroxidase, causing mitochondrial damage, free radical formation, and increased susceptibility to oxidative stress. This has yet to be investigated in FECD.

METHODS: Two SOD2-KD CEC lines, G1 and G2, were developed from B4G12 corneal endothelial cells (CECs) via CRISPR/Cas9. Knockdown was confirmed by quantifying SOD2 via microfluidic western blotting. Superoxide levels were evaluated with a MitoROS-580 assay. Mitochondrial bioenergetics, including basal respiration, ATP-associated oxygen consumption, proton leak, maximal respiration, spare respiratory capacity, and nonmitochondrial respiration were assayed via Seahorse XF96 extracellular flux analysis. All data were normalized to cell count and analyzed using a one-way ANOVA test. Cultured human patient derived TCF4 trinucleotide repeat expansion CECs, along with controls, were treated with 1 J/cm^2 UVA radiation. Protein was harvested from cell lysates, and SOD2 was purified using anti-SOD2 ELISA. SOD2 isolates treated with HNO3 and H2O2, and analyzed via ICP-MS for iron concentration. Statistics were performed using an unpaired t-test.

RESULTS: We found significant changes in superoxide levels and various markers of mitochondrial bioenergetics in SOD2-KD CECs compared to controls. Mean MitoROS levels of 0.5391 and 0.5348 for G1 and G2, respectively, were significantly higher ($p<0.0001$) than the levels measured for B4G12, which had a mean of 0.2978. Assessing mitochondrial bioenergetics, basal respiration was significantly decreased in both the G1 and G2 population ($p<0.0001$ and $p<0.05$), as was ATP production-coupled ($p<0.0001$ and $p<0.05$). Significant decreases in maximal respiration ($p<0.0001$) and spare respiratory capacity ($p=0.0002$) were only seen in the G1 population. Comparing UV-treated FECD cells to controls, respective means of iron-bound SOD2 were 784.6 ppm and 590.6 ppm ($P=0.2889$).

CONCLUSIONS: Reduced SOD2 expression in CECs results in increased levels of superoxide along with decreased levels of mitochondrial basal respiration, maximal respiration, spare respiratory capacity, and ATP production-coupled respiration in CECs. Iron-bound SOD2 protein in FECD CECs following UVA exposure is also increased compared to controls, albeit non-statistically significant due to high variability between samples. These results preliminarily indicate UV-mediated iron availability may increase transformation of SOD2 protein into prooxidant FeSOD2 in FECD CECs. Together, findings are consistent with the mitochondrial dysfunction and oxidative damage that has been noted in FECD, supporting the hypothesis that reduced or dysfunctional SOD2 may contribute to FECD pathogenesis.

Laser speckle flowgraphy shows reduced ocular blood flow in dry age-related macular degeneration (AMD)

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PURPOSE: Age-related macular degeneration (AMD) is a leading cause of irreversible blindness in the elderly, and there are a paucity of treatment options for the non-exudative form in part because the pathogenesis is not fully understood. The hemodynamic theory of AMD suggests poor blood flow is a risk factor. Unlike OCT-A which provides a binary measure of flow, laser speckle flowgraphy (LSFG) gives quantitative information on blood flow velocity. In this retrospective case-control study, we analyzed LSFG-derived ocular blood flow indices of patients with dry AMD versus age-matched healthy controls.

METHODS: Subjects with non-exudative AMD from the Iowa City VA Eye Clinic who had undergone LSFG scans were compared with age-matched controls. Blood flow quantified by MBR (mean blur rate) in arbitrary units (AU) was analyzed with the LSFG Analyzer software using binary threshold masking to separate it into chorioretinal flow (MT) and vascular flow (MV). A superpixel segmentation method was also used to quantify area of lowest choroidal blood flow (percentage area of MBR<5 AU). Student's T-test was used to see difference in MT and area of lowest flow between groups. Multivariate logistic regression was used to assess the association between MT and AMD diagnosis controlling for age, sex, smoking status, diabetes, hypertension, hyperlipidemia diagnosis, BMI and ocular perfusion pressure as covariates. Mixed effects linear regression was used to evaluate predictors of blood flow.

RESULTS: 24 eyes of 24 subjects with AMD and 21 eyes of 21 controls were included. Chorioretinal blood flow outside the major retinal vessels (MT) was lower in eyes with AMD compared with controls (5.3 ± 0.3 vs 7.9 ± 0.5, p< 0.0001 by t-test). Controlling for other subject and eye characteristics in the regression model, for every one-unit increase in MT, the odds ratio of AMD diagnosis was 0.36 in our population (95% CI 0.1-0.70, p=0.027). Intermediate and advanced AMD stage were significant predictors of lower blood flow after controlling for other covariates with mixed effects linear regression [intermediate: β -2.5±0.9, p=0.01; advanced: β -4.4±1.0, p=0.0003], and we found no relationship between blood flow and choroid vascularity index. Superpixel segmentation also showed a greater percentage of superpixels with lowest blood flow (MBR<5 AU) compared to controls (42 ± 5% vs 24 ± 5%, p=0.01 by t-test).

CONCLUSIONS: Eyes with AMD show a reduced ocular blood flow compared to controls that apparently exceeds the territory of retinal disease. Blood flow may serve as a potential target for AMD therapies in the future.

PLATFORM SESSION II

CHAIR: DR. NATALIA VERGARA

[1]1-12 PM

Modeling Alzheimer's disease retinopathy with human iPSC-derived retinal organoids

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PURPOSE: The neuropathological features that characterize Alzheimer's disease (AD), including the presence of beta-amyloid (Aß) plaques and intracellular neurofibrillary tangles of abnormally phosphorylated Tau proteins (pTau), are evidenced not only in the brain but also in the retina. This is likely to contribute to the visual manifestations of AD. In this context, hiPSC-derived retinal organoids offer new opportunities for AD modeling for drug development applications. Retinal organoids mimic the histoarchitecture of the native retina, have a consistent cellular composition, and are optically clear. Moreover, quantitative technologies have been established to facilitate assay design in these models without loss of their 3D structure. Thus, we set out to develop and characterize an hiPSC-derived retinal organoid model of AD that can be applied to the evaluation of pathophysiological mechanisms and to the validation of potential therapeutic drugs.

METHODS: Human iPSC-derived retinal organoids were generated from three healthy control (HC) and two familial AD (fAD) donors using the Zhong et al. (2014) protocol. Organoid structure, cellular composition and AD histopathology were assessed at three and six months of differentiation by immunofluorescence and histological staining, including retinal cell type-specific markers as well as Aβ, pathological pTau forms, and NIAD-4 staining for amyloid plaques. We also developed a NIAD-4 fluorescence-based assay for amyloid quantification in intact organoids using 3D-ARQ technology.

RESULTS: We found similarities in the cellular composition of AD and HC retinal organoids, confirming the validity of the models. However, pathological Tau hyperphosphorylation and Aß deposits were significantly increased in AD retinal organoids compared to HCs. Finally, we developed proof of concept of a quantitative assay for amyloid plaque detection that is amenable to translational research applications.

CONCLUSIONS: Our AD retinal organoid models mimic critical aspects of the histopathology of the human AD retina and constitute valuable tools for the screening and validation of candidate molecules with therapeutic potential.

Cis-regulatory Element Activity in Temporal Regulation of Retinal Cell Fate

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Purpose:The seven cell types of the mature vertebrate retina derive from a single pool of retinal progenitor cells (RPCs) and are born in a highly stereotyped, evolutionarily conserved and partially overlapping temporal birth order. The temporally-restricted specification of retinal cell fates is closely related to gene expression changes in RPCs as they shift their developmental competence. The transcription factors (TFs) associated with different retinal cell fates are well-characterized, but the noncoding DNA sequences where these TFs bind, act, and are regulated—cis-regulatory elements (CREs)—are only beginning to be uncovered.

METHODS: To overcome limitations with current methods for studying CREs, we have combined the assay for transposase-accessible chromatin (ATAC) with self-transcribing active regulatory region sequencing (STARR-seq) to directly and quantitatively measure genome-wide CRE activity in RPCs over the course of mouse retinal development. We generated a library of accessible DNA sequences in sorted RPCs from embryonic day 18 (E18) and cloned them into a self-transcribing enhancer reporter to read out CRE activity in the form of RNA transcripts. We then electroporated our library of accessible sequences from E18 RPCs into mouse retinal explants from either early (E14) or late (post-natal day 0, or P0) developmental ages.

RESULTS: We have identified almost 33,000 unique DNA sequences that are differentially active late in development—a period when late-born cell types such as rod photoreceptors, bipolar cells, and Müller glia are specified. By correlating the activity of these CREs with gene expression changes in RPCs, as well as cross-referencing our sequences with existing evolutionary conservation, chromatin conformation, and TF binding data sets, we have prioritized putative regulators of retinal cell fate specification for functional study. To biologically link our CRE activity to cell fate, we perform in vivo lineage tracing in RPCs to visualize whether a given active sequence confers cell type bias upon specification. We also perturb CRE activity in vivo via CRISPR interference (CRISPRi) to assess resulting changes in cell type proportions and retinal organization.

CONCLUSIONS: ATAC-STARR-seq, also referred to as high-resolution dissection of regulatory activity (HiDRA), is an unbiased method for measuring genome-wide CRE activity that provides a wealth of information regarding the complex regulatory network governing temporally-restricted cell fate in the vertebrate retina.

Reduced abundance of trabecular meshwork stem cells (TMSC) in human donor eyes with glaucoma

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PURPOSE: The trabecular meshwork (TM) of the eye is critical in maintaining aqueous humor outflow and intraocular pressure. The cellular density in the TM decreases with age and is particularly low in eyes with glaucoma. TM cells are thought to be derived from a population of stem cells, referred to as TM stem cells (TMSC), found in the 'insert zone' that is located anterior to the TM. We hypothesized that reduced cellularity of TM cells is due to loss of TMSC. In order to test this hypothesis, we determined the number of TMSC and TM cells in the eyes of young eye donors, as well as in glaucoma eyes and age-matched controls.

METHODS: Human eyes were obtained from young donors (n=3), healthy older donors (n=6), and those with glaucoma (n=7) and fixed in paraformaldehyde and embedded in paraffin. From each eye 5 sagittal section were obtained and incubated with antibodies directed against Nestin and ABCG2. The number of double positive cells in the insert zone and the trabecular meshwork was determined using fluorescence microscopy by an investigator blinded to the status of the sample. The cellularity of the TM was determined on the same sections by counting DAPI positive nuclei.

RESULTS: As expected, eyes of younger donors contained the largest number of TM cells (69.3 ±5.8 per section) while those of older, but healthy, donors contained more than glaucomatous eyes (59.8±6.5 vs 50.4±2.6; p=0.019). Likewise, we detected the largest number of TMSC in young eyes (5.84±1.58), significantly higher than in both healthy older eyes (2.79± 0.98, p=0.0012), or those with glaucoma (1.03± 0.16, p=0.0001 when compared to young eyes and p=0.012 when compared to age matched controls). Overall, we found a modest correlation between the number of TM cells and that of TMSC (R^2=0.53).

CONCLUSIONS: Our data indicate a clear decline in the number of TMSC with age and a further reduction in eyes with glaucoma. This finding is consistent with the notion that the degeneration or loss of stemness of TMSC is the cause of reduced TM cellularity which, in turn, is associated with the development of elevated intraocular pressure.

Elucidating the influence of microglia on retinal ganglion cells in a human pluripotent stem cell model

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PURPOSE: Glaucoma is the leading cause of irreversible blindness worldwide, affecting approximately 80 million people. In animal models of glaucoma, microglial activation has been associated with changes in morphology and proliferation, as well as the release of large amounts of inflammatory factors including tumor necrosis factor-α (TNF-α), interleukin IL-1β, and IL-6, which contribute to the neurodegeneration of retinal ganglion cells (RGCs). Due to major differences identified between rodent microglia and RGCs compared to humans, there is a critical need for novel human models that can be used to explore the cellular crosstalk between human microglia and RGCs and the role of this interaction in diseases such as glaucoma.

METHODS: In the current study, we differentiated both microglia-like cells (MGLs), RGCs and astrocytes from human induced pluripotent stem cells (iPSCs). Microglial activation was then induced using Lipopolysaccharide (LPS) and confirmed using morphological analyses, immunostaining, and cytokine/chemokine profile screening. Healthy and LPS-activated microglia were then co-cultured with RGCs for up to 3 weeks and the effects of microglia upon RGCs were assessed using measurements of soma size and neurite complexity. Finally, healthy and LPS-activated microglia were also co-cultured with astrocytes for up to two weeks, to assess if LPS-treated microglia can modulate astrocyte reactivity.

RESULTS: Results indicated that following activation via treatment with LPS, MGLs exhibit more round, amoeboid morphological features and increased the expression of MHC-II, characteristic of activated microglia. MGLs also released significantly elevated levels of inflammatory cytokines including IL-6, IL-8, IL-1B and TNFa. To determine the effects of microglia upon RGCs, including both quiescent and activated MGLs, we then established novel co-cultures of MGLs and RGCs. These studies revealed that activated MGLs reduced RGC neurite complexity and soma size compared to healthy microglia, suggesting a contribution of microglia activation to RGC neurodegeneration. Additionally, when co-cultured with astrocytes, LPS-activated microglia promoted astrocyte reactivity.

CONCLUSIONS: Taken together, the current study establishes the first known study to develop a human cellular model that allows for the examination of cellular interactions

between human microglia, astrocytes and RGCs, including the study of microglial contributions to RGC neurodegeneration and neuroinflammation.

PLATFORM SESSION III

CHAIR: DR. SHEILA BAKER

1-2 PM

KCNV2 Retinopathy: from eyeballs to atoms

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PURPOSE: KCNV2 retinopathy is an enigmatic congenital retinal degeneration affecting children. It is a form of early-onset macular degeneration with aberrant electrical signaling that is diagnosed using ERG. Mutations in the gene KCNV2 were identified as the cause of the disease at the turn of the century. KCNV2 encodes a voltage-gated ion channel, Kv8.2. Kv8.2 only forms functional channels by assembling with Kv2.1. The heteromeric Kv2.1/Kv8.2 channel regulates photoreceptor membrane potential. We developed mice lacking Kv8.2 expression to investigate the molecular mechanisms of Kv2.1/Kv8.2 function and to model KCNV2 retinopathy.

METHODS: We generated Kv8.2 KO mice using CRISPR. Kv8.2 KO mice were bred to a mouse strain with an all-cone retina (NRL KO, RPE65 R91W+/-) to generate Conefull: Kv8.2 KO. Retina function was analyzed using ERG. Retina structure was analyzed using OCT, histology, and transmission electron microscopy. Recombinant Kv2.1-T1 domain was purified from E. coli and the X-ray crystal structure solved by molecular replacement. AlphaFold2 was used to create a hetero-tetrameric Kv2.1/Kv8.2-T1 model.

RESULTS: A series of ERG tests demonstrate that the Kv8.2 KO mice phenocopy the features of KCNV2 retinopathy. Rod-driven signaling is characterized by a reduced amplitude and square a-wave, the b-wave is delayed and has a supernormal amplitude, and the c-wave amplitude is reduced. Cone-driven signaling elicited by flickering light in both Kv8.2 KO and Conefull: Kv8.2 KO is reduced. Kv8.2 KO mice undergo a mild retinal degeneration, 30% loss at 10 months of age, but no loss of cones, measured out to 1 year of age. Degeneration in Conefull mice is accelerated in Conefull:Kv8.2 KO. Most missense mutations associated with KCNV2 retinopathy are found in the T1 tetramerization domain which indicates that channel assembly is particularly vulnerable. To investigate the molecular mechanism of channel assembly we were able to solve the X-ray crystal structure of the Kv2.1-T1 domain and modeled in a Kv8.2-T1 subunit. This revealed that two features adding stability to the Kv2.1-T1/T1 interface are inter-

CONCLUSIONS: Kv8.2 KO mice phenocopy the aberrant electrical signaling characteristic of KCNV2 retinopathy. The defective cone signaling is independent of rods, but the presence of rods protects cones from degenerating. Additional molecular studies are needed to learn how assembly of Kv8.2 with Kv2.1 is regulated. Meanwhile, our mouse models could be used for pre-clinical testing of gene or drug therapies for KCNV2 retinopathy.

Novel nrf2 mutant zebrafish fail to regulate target genes and are susceptible to light-induced photoreceptor death.

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PURPOSE: Oxidative stress-provoked photoreceptor degeneration is a critical factor in retinal disease pathologies such as retinitis pigmentosa. NF-E2-related factor 2 (NRF2; NFE2L2) is an important transcriptional regulator for redox homeostasis. In stressed conditions, Nrf2 translocates to the nucleus to bind and activate antioxidant response elements, which upregulate target antioxidant genes. The increased production of antioxidants break-down reactive oxygen species, allowing cells to return to redox homeostasis. Thus, nrf2 is an attractive target for treating retinopathies like retinitis pigmentosa. Danio rerio have two orthologs of the human NRF2 gene, nrf2a and nrf2b, but only nrf2a has been knocked out. A complete nrf2 mutant will provide a platform to investigate the role of oxidative stress in retinal diseases and inform therapeutic strategies. The purpose of this study is to establish and validate a novel nrf2 mutant zebrafish model where both nrf2a and nrf2b are knocked out.

METHODS: The CRISPR-Cas9 system was used to produce a large deletion in nrf2b. The nrf2b mutants generated were bred with an established nrf2a mutant line, nrf2afh318 (Goessling lab). The double homozygous mutant offspring (nrf2) were confirmed with sequencing. A light damage assay (LDA) was used to induce retinal cell death by having five days post fertilization (dpf) fish exposed to normal or excessive light conditions for three days. To evaluate the susceptibility of photoreceptors to light damage, pyknotic nuclei and TUNEL positive cells in the outer nuclear layer (ONL) were quantified in wildtype and nrf2 mutants (8dpf, n=15). A qPCR assay was used to determine if nrf2 mutants have reduced activation of antioxidant pathways in LDA conditions compared to wildtype fish (n=3, 5 biological replicates). Three nrf2 target genes were measured, a glutamate-cysteine ligase (gclc), a NAD(P)H dehydrogenase (nqo1), and heme oxygenase-1 (hmox1a).

RESULTS: A two-way ANOVA showed that after light damage, nrf2 mutants have significantly higher pyknotic (21.3 ± 1.37, $p = 0.001$) and TUNEL positive cell count (1.5±0.55, $p = 0.018$) in the ONL compared to wildtype fish (10.2±1.18; 0.73±0.17). Furthermore, the expected upregulation of nrf2 target genes in light damage conditions was not induced in nrf2 mutants. qPCR results indicated a significant fold change of gclc, nqo1, and hmox1a expression in light damaged wildtype fish (48.2±10.5; 91.9±41.3; 7.68±1.22, $p = 0.03$) while gene expression remained at basal levels in light damaged nrf2 mutants (1.27±0.17; 0.43±0.07; 0.40±0.07).

CONCLUSIONS: The data suggests nrf2 mutants fail to regulate downstream target genes in LDA conditions and are sensitive to light damage-induced photoreceptor death. To validate heightened oxidative stress in nrf2 mutants after LDA conditions,

retinal antioxidant activity will be assessed using redox-sensitive GFP reporter lines. In future studies, nrf2 mutants will be applied to a model of Usher Syndrome subtype 2A (USH2A; Usherin), a rare retinopathy that causes progressive photoreceptor death. Oxidative stress may be a factor involved in the pathogenesis, thus, the loss of Nrf2 activity may increase susceptibly for further cell death in the USH2A mutant zebrafish model.

Contribution of CD4 and CD8 cells to retinal ganglion cell loss in glaucoma

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PURPOSE: Loss of retinal ganglion cells (RGC) is the ultimate cause of vision loss in glaucoma. Although elevated intraocular pressure (IOP) has been identified as a main risk factor for the development of glaucoma, disease progression is likely due to multiple pathomechanisms. Previous studies in our laboratory have demonstrated that adoptive transfer of CD3+ cells from glaucomatous mice into healthy recipients results in progressive loss of RGC. Conversely, RAG mice lacking T or B cells, are profoundly protected from glaucomatous damage caused by elevated IOP. These findings indicate that adaptive immune responses are one of the factors leading to vision loss in glaucoma. The current study was carried out in order to determine the functional contribution of CD4+ and CD8+ T cells in this process.

METHODS: Elevated IOP was induced in the eyes of C57Bl/6J controls, Cd4 knockout (KO), and Cd8 KO mice through intracameral injection of Ad5.MyocY437H (n=25/group). IOP was measured weekly using rebound tonometry. Visual function was determined every month by measurements of the optokinetic response (OKR). RGC loss was assessed in whole-mounted retinas following immunohistochemical labeling of RGC with antibodies directed against RBPMS. After 4 months of elevated IOP, spleens were harvested, homogenized, and transferred into RAG recipient mice by intraperitoneal injection.

RESULTS: After 4 months of elevated IOP, the OKR in controls had decreased by 0.097 cycle/degree (c/d), whereas visual acuity in Cd4 KO decreased by 0.050 ($p=0.03$) and by 0.068 c/d in *Cd8* KO ($p=0.26$). RGC density decreased in all groups with elevated IOP ($p<0.0009$). Losses in neither *Cd4* nor *Cd8* KO were significant when compared to control mice. RAG mice receiving adoptive transfers of splenocyte preparations from these groups also developed RGC damage. 4 months after transplantation, OKR responses in RAG which had received control splenocytes decreased by 0.069 c/d ($p=0.032$), whereas those that received *Cd4* KO or *Cd8* ko splenocytes decreased by 0.048 c/d ($p=0.21$), or 0.083 ($p=0.009$), respectively. RGC density in recipient mice decreased from 3,404 cells/mm^2 in naïve RAG mice to 2,865 cells/mm^2 in control recipients, 2,938 cells/mm^2 in *Cd4* KO recipients, and 2,544 cells/mm^2 in *Cd8* KO recipients. No differences between *Cd4* or *Cd8* KO were found when compared to control recipients.

CONCLUSIONS: Neither absence of CD4+ or CD8+ cells resulted in robust RGC protection in donor or recipient mice. This suggests either compensatory mechanisms in

Cd4 and *Cd8* KO mice, or that other splenocyte cell populations contribute stronger to RGC loss than appreciated.

Validation of the Iowa Head-Mounted Open-Source Perimeter

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PURPOSE: Visual field testing is used extensively in the diagnosis and surveillance of many ocular diseases. Projection-based perimeters are most commonly used and can cost upwards of $30,000. This high price point restricts access to visual field testing, particularly for individuals in low-income areas, such as underdeveloped countries. The purpose of this study is to assess the validity of visual field results from the Iowa Head-Mounted Display (HMD) Open-Source Perimeter, an open-source virtual reality headset programmed for visual field testing. With hardware costs of approximately $150, this system has the potential to improve perimetry access.

METHODS: We tested 20 healthy and 9 glaucoma patients on the HMD and Octopus 900 perimeters using the Open Perimetry Interface platform with size V stimuli, a custom grid spanning the central 26 degrees of the VF, and a ZEST thresholding algorithm. Historical data from the Humphrey Field Analyzer (HFA) was also analyzed. Repeatability was analyzed with the repeatability coefficient (RC), and VF defect detection was determined through side-by-side comparisons.

RESULTS: The pointwise RC was 2.6 dB and 3.4 dB for the HMD and Octopus 900 perimeters in ocular healthy subjects, respectively. Likewise, the RC was 4.2 dB and 3.5 dB in glaucomatous patients. Limits of agreement between the HMD and Octopus 900 perimeters were ±4.6 dB (mean difference 0.4 dB) for healthy patients and ±8.9 dB (mean difference 0.1 dB) for glaucomatous patients. Retrospective analysis showed pointwise RCs on the HFA2 perimeter to be between 3.4-3.7 dB for healthy patients and 3.9-4.7 dB for glaucoma patients. VF defects were similar between the HMD and Octopus 900 for glaucoma subjects.

CONCLUSIONS: The Iowa Virtual Reality Headset Open-Source Perimeter accurately reproduces visual field defects, is as repeatable as the Octopus 900 perimeter, and is a more portable and less expensive alternative than traditional perimeters.

POSTER PRESENTATIONS

EVEN NUMBERED POSTERS : PRESENTING 10-11 AM

ODD NUMBERED POSTERS: PRESENTING 2-3 PM

Peter Sanchez	Poster 01	Umbilical amnion for posterior lamellar eyelid reconstruction and anopthalmic socket reconstruction: a retrospective chart review
Antonia Amidon	Poster 02	Inhibition of baxa or baxb does not rescue retinal ganglion cell death in atoh7 loss of function Danio rerio
Jeffrey Anders	Poster 03	Inhibition of HIF-1 Signaling Reduces Visual Impairment in a Mouse Model of Multiple Sclerosis-like Optic Neuritis
Nickolas Boehme	Poster 04	Retinal and optic nerve damage in a mouse model of blast-mediated traumatic brain injury
Erin Capper	Poster 05	Utilization of Ketogenic Diet Worsens Visual and Motor-Sensory Deficits in An Animal Model of Multiple Sclerosis
Pavithra Chinna Swamy	Poster 06	Structural and Biochemical Investigation of NRL and CRX Cooperativity
Michael Donohue	Poster 07	In vivo investigation of mia2/mia3 in protein transport within zebrafish photoreceptors
Ismael Hernandez Nunez	Poster 08	Examining the role of the TET proteins and DNA demethylation in retinal cell fate determination
Shivangi Inamdar	Poster 09	Labeling and enrichment of retinal proteins using non-canonical amino acids
Rishika Joshi	Poster 10	Effect of 670 nm photobiomodulation on angiogenic and inflammatory signaling pathways in cultured cell model of diabetic retinopathy
Grace Lee	Poster 11	Corneal sensitivity in chronic orbital pain treated with peripheral trigeminal nerve blocks
Brianna Lobeck	Poster 12	Pre-clinical testing of two candidate vectors for treating Bardet-Biedl Syndrome type 10 with subretinal gene therapy
Serena Patel	Poster 13	Methylenetetrahydrofolate reductase expression in human diabetic retinas
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Brynn Schubert	Poster 15	Potential Treatment of Elevated Homocysteine on

		Retinal Pigmented Epithelial Cells
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Salma Hassan	Poster 19	A Dose Response Study for Subretinal Gene Therapy for Treating X-Linked Juvenile Retinoschisis in a Retinoschisin 1 Knockout Mouse Model
Shaylah McCool	Poster 20	5xFAD Mouse Model and the Early Visual System
Noriyoshi Takahashi	Poster 21	Automated Optic Disc Finder and Segmentation Using Deep Learning for Blood Flow Studies in the Eye
Oliver Gramlich	Poster 22	Complement Dependent Cytotoxicity Drives Retinal Ganglion Cell Loss in a Novel In Vivo Model of Neuromyelitis Optica
Sara Mayer	Poster 23	Antioxidant treatment suppresses retinal defects in Drosophila models of SNRNP200-associated Retinitis Pigmentosa
Jui-Kai Wang	Poster 24	Visualization and Quantification of the Spatial Patterns of Edema and Atrophy in Non-Arteritic Anterior Ischemic Optic Neuropathy (NAION) Over Time Based on Deep-Learning Variational-Autoencoder
Hend Al-Kaylani	Poster 25	Role of SIN3A dysregulation in neural crest differentiation and Witteveen-Kolk syndrome

Poster #2

Inhibition of baxa or baxb does not rescue retinal ganglion cell death in atoh7 loss of function Danio rerio

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PURPOSE: Retinal ganglion cells (RGCs) transmit all visual information to the brain as their axons form the optic nerve and are thus essential for vision. Studies have demonstrated that the baxa and baxb genes are mediators of apoptosis in zebrafish, mimicking Bax function in mammalian species. In mice, RGC cell death can be rescued by inhibition of apoptosis through the loss of the pro-apoptotic Bax gene, however it is unknown if this mechanism is shared by zebrafish.

METHODS: Translation blocking morpholinos (MOs) or crRNA/Cas9 targeting atoh7, baxa, and or baxb, were used to inhibit protein or gene function, respectively, through injection into embryos of either atoh7(lakritz), baxa, or baxb genetic mutants. Survival of RGCs were assessed with laser scanning confocal microscopy using the Isl2b3:nGreenLanternPEST transgene background, or via wholemount immunostaining of the pan RGC marker Rbpms2b.

Results Injection of both the previously published atoh7 MO and dual cRNAs targeting atoh7 resulted in a complete loss or reduction Isl2b3:nGreenLanternPEST or Rbpms2b positive RGCs compared to uninjected controls. Preliminary results suggest loss of baxa or baxb alone does not rescue the RGC cell death induced by loss of atoh7 function.

CONCLUSIONS: Baxa or baxb are not solely responsible for the loss of RGCs observed in atoh7 loss of function zebrafish. Further experiments will need to be repeated to gain a larger sample size and assess the possibility of genetic compensation between baxa and baxb in inducing RGC cell death in atoh7 mutants.

Poster #4

Retinal and optic nerve damage in a mouse model of blast-mediated traumatic brain injury

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PURPOSE: Visual problems are often present in traumatic brain injuries (TBI) acutely and chronically. Thinning of the retina was observed in different types of TBI, in particular a decrease in the thickness of the retinal ganglion cell (RGC) layer and/or of the RNFL. Our recent work using a mouse model of blast-induced TBI indicates that RGCs may be particularly prone to damage associated with over-pressure waves. To understand the mechanism of injury we characterized cellular and tissue changes in the retina and optic nerve in this mouse model.

METHODS: In a custom-built blast chamber, mice were exposed to an overpressure wave (20 PSI) directed to the head. Optical coherence tomography (OCT) was used to characterize the retinal structure in vivo. The number of RGCs and the total number of cells in the RGC layer were determined using an RGC specific antibody and hematoxylin and eosin staining, respectively, in whole mounted retinas. Optic nerve damage was assessed using histological stains and antibodies specific for glia.

RESULTS: The RGC complex thickness was decreased in injured mice compared to sham. The number of RGCs decreased significantly as early as one week after injury, along with the total number of cells from the RGC layer, while the retinal area remained constant. The analysis of optic nerves stained post-injury showed a decrease in number of myelinated axons and an increase in number of glial cells.

CONCLUSIONS: In this model, exposure to an overpressure wave damaged both the retina and the optic nerve, with RGC loss, and glial activity in the optic nerve. By establishing the pathophysiology of retinal and optic nerve damage and the time-line of these changes after injury we are enabling the development of improved clinical diagnosis and treatment of traumatic brain injuries.

Poster #6

Structural and Biochemical Investigation of NRL and CRX Cooperativity

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PURPOSE: CRX and NRL are two key transcription factors involved in the gene regulation during photoreceptor differentiation and homeostasis. While CRX is essential for the differentiation of postmitotic photoreceptor precursors to the photoreceptor cells, NRL determines rod cell fate. During photoreceptor development, CRX and NRL cooperate functionally and physically via direct protein-protein interaction and regulate the transcription of various genes. Despite of extensive studies, there is no clear understanding of precise molecular mechanisms that determine CRX- and NRL-mediated expression of several rods and cone genes during development and in response to signaling pathways. The aim of this study is to elucidate molecular mechanisms of the CRX/NRL function based on the crystal structures and to determine structural basis for synergy and specificity of NRL and CRX interaction in photoreceptor gene regulation.

METHODS: CRX homeodomain protein was purified and attempted to determine solution structure by small angle X-ray scattering (SAXS). Attempts were also made to crystalize CRX homeodomain with rhodopsin promoter. Binding affinity of NRL and CRX with their respective response elements were determined by fluorescence anisotropy. NRL and CRX cooperativity was investigated by electrophoretic mobility shift assay and single molecule-total internal reflection microscopy.

RESULTS: We have solved the crystal structure of CRX in complex with its cognate response element from rhodopsin promoter. The structure clearly shows that CRX and DNA interacts with 2:1 stoichiometry and causes DNA bending.

CONCLUSIONS: The complex structure shows unusual stoichiometry which was validated by SEC-MALS-SAXS and single molecule-total internal reflection microscopy. Electromobility shift assay suggests that CRX increases the affinity between NRL and DNA. However, presence of NRL changes the stoichiometry of CRX binding to its response element in rhodopsin promoter. Our current work set the ground for the future studies on the mechanism of NRL-CRX cooperativity on other promoters and mechanism of transcription regulation by the two transcription factor.

Poster #8

Examining the role of the TET proteins and DNA demethylation in retinal cell fate determination

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PURPOSE: DNA methylation is a dynamic process by which methyl groups are added and removed from cystine residues for the regulation of gene expression. During development, DNA methylation functions to maintain stem cells properties or stimulate cell differentiation. Active DNA demethylation is driven by the tet-eleven translocation (Tet) methylcytosine dioxygenase proteins. In the zebrafish, Tet2 -/-; Tet3 -/- mutants retinas result in reduced retinal ganglion cell axonal migration and impaired differentiation of photoreceptors. In this study, we seek to identify the functional role and potential redundancy of the Tet proteins in cell-fate determination within the developing mammalian retina.

METHODS: Postnatal day (P) P21-P24 and 6 weeks old heterozygous and Tet conditional knockouts (cKO) were generated using the Chx10-Cre line. Retinal morphology and visual function were examined using hematoxylin and eosin staining (H&E), immunohistochemical approaches and visual function assays. The consequence of Tet protein overexpression on retinal cell fate specification was determined through ex vivo electroporations of P0 CD1 mice Statistical analyses were performed with GraphPad Prism v9.

RESULTS: Morphological assessments of Tet mutant retinas indicate Tet1/2/3 triple cKOs result in a disruption of retinal structure, and the absence of outer segments at P21. Immunohistological analyses determined a significant reduction in the number of horizontal cells (Calbindin+) and a significant increase in amacrine cells (Pax6+), Müller glia cells (Lhx2+), and microglial cells (Iba1+) in Tet1/2/3 cKO retinas. Additionally, Tet1/2/3 cKO resulted in a significant increase in the number of cones (RXRgamma+; cone-arrestin+), with cone nuclei mislocalized to the basal portion of the outer nuclear layer (ONL). Changes in cell type proportions resulted in decreased amplitudes of dark-adapted A and B waves, and the light-adapted B wave in the Tet1/2/3 cKOs. Overexpression experiments resulted in small but significant changes in cell fate specification after Tet overexpression.

CONCLUSIONS: Loss of active DNA demethylation within the developing mouse retina results in failure of photoreceptor function in the adult animal. Future experiments seek to identify the mechanisms by which the Tet proteins control photoreceptor cell fate specification and differentiation.

Poster #10

Effect of 670 nm photobiomodulation on angiogenic and inflammatory signaling pathways in cultured cell model of diabetic retinopathy

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PURPOSE: The pathophysiology of DR is complex, involving mitochondrial dysfunction, oxidative stress, and vascular degeneration. Anti-VEGF drugs are used to reduce vascular proliferation. Photobiomodulation treatment (PBMt) with far-red (670 nm) light is a promising therapy for DR. In an in vitro model of DR, we showed that high glucose exposure disrupts mitochondrial function increases ROS and initiates a signaling cascade leading to NFkB activation and increased ICAM-1 and VEGF production. PBM treatment attenuated oxidative stress and mitochondrial dysfunction, reduced NFkB and decreased production of ICAM-1. However, PBM did not reduce VEGF, leading us to speculate that other signaling pathways may be involved in the action of PBM. This study tested the hypothesis that 670nm PBM treatment will enhance the expression of two key anti-angiogenic and anti-inflammatory factors, thrombospondin-1 and transforming growth factor β in this Muller glia cell model of diabetic retinopathy.

METHODS: Müller glial cells play a primary role in the development and progression of DR due to a shift in their physiology from an anti-inflammatory to a pro-inflammatory state. Rat retinal Müller cells (rMC-1) were cultured in normal (5 mM) or high (25 mM) glucose media to simulate normoglycemia and hyperglycemia. Cultures were treated with a 670 nm light using a light emitting diode (LED) array at a dose of 4.5 J/cm^2 (25 mW/cm^2 for 180 seconds) or no light (sham) for 3 or 4 days. Assays were conducted to measure thrombospondin-1 (TSP-1), an anti-angiogenic extracellular matrix-associated glycoprotein and transforming growth factor beta (TGF-β), a multifunctional anti-angiogenic and anti-inflammatory cytokine.

RESULTS: High glucose treatment had no effect on TSP-1 concentrations in MGCs. In contrast, PBM treatment produced a two-fold increase in MGC's exposed to high glucose ($p = 0.046$). Further studies are needed to confirm these findings. We were unable to visualize TGF-β due to serum interference.

CONCLUSIONS: The ability of 670nm light treatment to attenuate early molecular changes in this in vitro high glucose model of DR suggest that PBM treatment has the potential to mitigate early deleterious effects in DR by modulating inflammatory signaling and diminishing oxidative stress. Further studies are needed to fully characterize the effects of PBM treatment on signaling pathways involved in the pathogenesis of diabetic retinopathy.

Poster #12

Pre-clinical testing of two candidate vectors for treating Bardet-Biedl Syndrome type 10 with subretinal gene therapy

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PURPOSE: Bardet-Biedl Syndrome type 10 (BBS10) is an autosomal recessive disease characterized by retinal degeneration leading to severe vision loss. Mutations in at least 29 genes can cause BBS; BBS10 accounts for about 40% of cases. Subretinal gene augmentation therapy to for BBS10 has shown promise in slowing vision loss in a mouse model of BBS10. The choice of promoter used to drive target gene expression can affect the efficacy of therapy. In this study, BBS10 gene expression is driven by two distinct promoting regions: chicken beta-actin (CAG), a general promoter, and rhodopsin kinase (RK), a photoreceptor-specific promoter. The purpose of this study is to investigate the toxicity and efficacy of these two candidate viral vectors, AAV8-CAG-hBBS10 and AAV8-RK-hBBS10.

METHODS: A Bbs10 knockout (Bbs10 -/-) mouse model was maintained both on an SV129 background and a mixed genetic background. The human BBS10 gene was cloned into shuttle plasmids driven by the RK promoter and the CAG promoter, respectively. These plasmids were packaged into an AAV2/8 viral capsid for in vivo gene delivery. To assess toxicity, wild-type (WT) and heterozygous (HET) mice of both genetic backgrounds were subretinally injected with 2 µL of AAV8-CAG-hBBS10 or AAV8-RK-hBBS10 at 4×1010 vg/µL (digital droplet PCR), for a total dose of 8×1010 vg/eye. To determine efficacy, Bbs10-/- mice received either the AAV8-CAG-hBBS10 or the AAV8-RK-hBBS10 vector at 8×108, 8×109, or 8×1010 vg/eye. Electroretinography (ERG) was used to assess retinal electrical response and was completed at 1-, 2-, 3-, and 5-months post injection (PI). Optical coherence tomography (OCT) was used to assess retinal structure at 1-, 3-, and 5-months post injection (PI). Data quantification was performed using ImageJ.

RESULTS: Toxicity: After injection of AAV8-RK-hBBS10, thicknesses of the outer nuclear layer (ONL) in WT/HET mice were comparable to those in eyes treated with buffer ($p > 0.95$) or untreated eyes ($p > 0.94$). For WT/HET mice receiving AAV8-CAG-hBBS10, a strain-specific effect was observed. After receiving 8×1010 vg/eye of AAV8-CAG-hBBS10, ONL thicknesses in WT/HET mice on the SV129 background displayed no difference from those receiving the dilution buffer ($p=0.998$). In contrast, WT/HET mice on the mixed background had significantly thinner ONLs than buffer-treated or untreated eyes as early as 1-month PI after receiving this dose. Efficacy: At 2-month PI, Bbs10-/- mice treated with AAV8-RK-hBBS10 had higher ERG amplitudes in light-

adapted but not dark-adapted ERGs. At 3-months PI, mice treated with AAV8-CAG-hBBS10 possessed higher b-waves in their dark-adapted ERGs, whereas b-wave results in untreated Bbs10-/- mice became nonrecordable; improvements were not seen in the light-adapted ERG.

CONCLUSIONS: Toxicity Study: A high-dose administration of the AAV8-RK-hBBS10 vector was well-tolerated in the eyes of WT/HET mice. In contrast, AAV8-CAG-hBBS10 caused strain-specific retinal degeneration. Overexpression toxicity may be a factor underlying the degeneration. The reason for the strain-specific response is being explored. Efficacy Study: Mice treated with AAV8-RK-hBBS10 do not exhibit better dark-adapted ERG amplitudes; however, cone activity improved in a dose-dependent manner. Mice treated with AAV8-CAG-hBBS10 showed a slower loss of b-wave amplitudes over time compared to untreated mice, revealing conserved bipolar cell activity. Overall, AAV8-RK-hBBS10 had a superior safety profile and elicited improvement in retinal function in a mouse model of BBS10.

Poster #14

Development of a three-dimensional organoid model to explore early retinal phenotypes associated with Alzheimer's disease

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PURPOSE: Alzheimer's disease (AD) is a progressive neurodegenerative disorder characterized by the accumulation of Aβ plaques and neurofibrillary tangles, resulting in neurodegeneration. The retina is an extension of the central nervous system within the eye, sharing many structural similarities with the brain, and previous studies have observed AD-related phenotypes within the retina. Human pluripotent stem cells (hPSCs) can effectively model some of the earliest manifestations of disease states, including those affecting the retina, yet early AD-associated phenotypes have not yet been examined. Thus, the current study focused upon the differentiation of hPSCs into retinal organoids for the analysis of AD-associated alterations.

METHODS: hPSCs from both AD as well as healthy controls were used in all studies, and their pluripotency as well as the presence or absence of AD-associated gene variants was validated. Subsequently, cell lines were directed to differentiate to yield retinal organoids following established methods. Upon acquisition of retinal organoids, we assesed for characteristic features of AD pathology, including changes in the levels of pathological Aβ42 as well as phosphorylated tau protein. Retinal organoids with AD-associated mutations were then transcriptionally profiled to further identify other alterations that may be leveraged as early indicators of AD pathology.

RESULTS: Results demonstrated the robust differentiation of retinal organoids from both familial AD and unaffected control cell lines. AD retinal organoids also exhibited characteristic pathological features, including an elevation in the Aβ42:Aβ40 ratio in conditioned medium, as well as a significant increase in pTau protein in AD retinal organoids. Transcriptional analyses further demonstrated the differential expression of many genes and cellular pathways, particularly those associated with synaptic dysfunction.

CONCLUSIONS: The current study demonstrates the ability of retinal organoids to serve as a powerful model for the identification of some of the earliest retinal alterations associated with AD, thereby validating the potential use of retinal organoids as a model

system to explore early alterations within an easily accessible region of the central nervous system, along with important implications for the early diagnosis of AD.

Poster #16

Microglia In the Visual Thalamus Respond to Glaucomatous Neurodegeneration

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PURPOSE: Glaucoma causes blindness by damaging retinal ganglion cells (RGCs) until they are irreversibly lost. Chronically high eye pressure (ocular hypertension; OHT) is glaucoma's only modifiable risk factor and has previously been shown to initiate a loss of RGC axon terminals in retinorecipient brain regions, including the visual thalamus (dorsolateral geniculate nucleus; dLGN). During development, the dLGN undergoes a period of synaptic pruning whereby microglia- the innate immune cells of the brain- remove components of excess synapses via phagocytosis. Because we hypothesize that OHT may be triggering the removal of RGC axon terminals via similar mechanisms, we sought to investigate whether microglia are involved in removing synapses in the glaucomatous dLGN.

METHODS: To this aim, we investigated whether OHT triggers microglial polarization and molecular-tagging of dLGN synapses in a mouse model of inherited glaucoma (DBA/2J; D2) and its strain-matched control (DBA/2JGpnmb+). We longitudinally monitored intraocular pressure (IOP) during the living phase, then used fixed tissue to examine dLGN-targeting RGC axon terminals at timepoints corresponding to pre- (4mo), early- (9mo), and progressive glaucoma (12mo). Correlations were made using the IOP integral (AUC in mmHg*days) for each eye separately.

RESULTS: D2s showed an IOP-dependent reduction (R^2 = 0.31; $p<0.001$) in RGC terminals immunolabeled for vesicular glutamate transporter 2 (vGlut2+), while no age- or IOP-related loss was observed in controls ($p = 0.52$; $p = 0.850$). A subset of tissues was stained for complement protein C1q, which tags immature retinogeniculate synapses for removal during development. An intensity analysis revealed that C1q was elevated by 9-months in glaucoma and exaggerated by 12-months: this increase was IOP-dependent (R^2 = 0.48; $p < 0.0001$) and associated with vGlut2+ loss (R^2= 0.40; $p< 0.001$). Pre-glaucoma C1q expression was consistent with the low, unchanging levels that were detected at all timepoints in controls ($p = 0.28$), suggesting that complement-mediated synapse elimination may be in effect. Morphological analysis of Iba1+ cells (microglia/macrophages) revealed negative associations between OHT and ramification features (IOP explained ~ 30% of branching variability (R^2= 0.30, $p<0.0001$).

CONCLUSIONS: These data suggest that Iba1+ cells in the visual thalamus may be polarized towards a synapse-removal state in the context of glaucoma. Moreover, the

glaucoma-specific increase in C1q-expression in the dLGN may be evidence that chronic IOP elevations are aberrantly reactivating parallel mechanisms to those involved in developmental pruning- future work will address these details.

Poster #18

The effects of trisomy on retinal thickness and retinal ganglion cell count in a postnatal Ts65Dn mouse model of Down syndrome

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PURPOSE: Down syndrome (DS) is a genetic condition that is caused by the triplication of human chromosome 21 and presents with a variety of neurological phenotypes, including hypocellularity in the brain, and various ocular phenotypes, including an increased risk of cataracts, increased risk of refractive errors, and increased retinal thickness. The Ts65Dn mouse model of DS presents with a number of similar phenotypes to those with DS including decreased brain volume, decreased visual acuity, and increased retinal thickness. Victorino et al. 2020 found an increase in retinal thickness in P17 Ts65Dn mice. Laguna et al. 2013 showed that adult Ts65Dn mice had a thicker retina, along with an increased cellularity in the retinal ganglion cell (RGC) layer. To develop a treatment plan to normalize ocular phenotypes, the time point in which the trisomic mice deviate from the euploid mice must be determined. We hypothesize that retinal thickness and RGC count will be increased at P15 in Ts65Dn mice.

METHODS: Retinal cryosections from male and female trisomic and euploid Ts65Dn mice at P15 were fluorescently labeled for RGCs and bipolar cells via cryo-immunofluorescence. The retinas were measured for total retinal thickness and RNA-binding protein with multiple splicing (RBPMS) positive cells in the RGC layer. Measurements for total retinal thickness were taken at 200μm increments from the optic disc. RBPMS positive cells were counted in the region of the RGC layer 200μm and 400μm from the optic disc.

RESULTS: There were no genotypic or sex effects in either total retinal thickness or RBPMS positive cells at P15 in male and female Ts65Dn mice.

CONCLUSIONS: These preliminary results indicate that the lack of genotypic or sex effect in both total retinal thickness or RBPMS positive cells means that this age could be a good starting point for a treatment normalize total retinal thickness and RBPMS positive cells at later ages. These findings are different than what was seen previously at P17 in the Ts65Dn model in Victorino et al. 2020, in which there was an increased in total retinal thickness in the trisomic mice compared to the euploid mice. Differences in measuring technique, optical coherence-tomography as compared to measuring 2D images of retinal slices, as well as a difference in the strain of Ts65Dn, 005252 as compared to 001924, could be an alternate reason as to the differences between the studies.

Poster #20

5xFAD Mouse Model and the Early Visual System

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PURPOSE: Alzheimer's disease (AD) is the most prevalent neurodegenerative disorder and form of dementia diagnosed worldwide. AD is characterized by a loss of memory and cognitive impairment due to the formation of amyloid beta (Aβ) plaques and neurofibrillary tangles (NFT), and ultimately, atrophy of the brain. It is known that AD impacts the visual system; however, the mechanisms by which it does this are not well understood. The global impact of AD leads us to study the structure and function of the retina and the dorsal lateral geniculate nucleus (dLGN), the visual thalamus. The dLGN is the primary target of retinal projections for image-forming vision as well as relaying information from the retina to the cortex. The purpose of this project was to determine mechanisms that underly visual deficits in Alzheimer's disease (AD) by investigating the hypothesis that Aβ pathology leads to neuronal and synaptic degeneration in the retina & dLGN.

METHODS: The 5xFAD mouse model develops an AD-like phenotype, specifically the formation of Aβ plaques, and will be used to test our hypothesis along with C57BL/6J mice used as controls. To examine whether retinal function is altered in AD, electroretinogram (ERG) recordings were performed. Immunohistochemistry of vGlut2 was performed to determine number of retinal ganglion cell (RGC) axon terminals in the dLGN. Cell fills of thalamocortical (TC) neurons followed by 2-photon imaging was utilized to examine dendritic structure in the dLGN. Miniature excitatory postsynaptic current (mEPSC) recordings were used to determine function of the retinogeniculate synapses. Optomotor response (OMR) was used to show reflexive visual behavior.

RESULTS: The 5xFAD model was successfully validated after analyzing Aβ plaque density in the dLGN which presented with a high burden of Aβ plaques compared to C57BL/6J controls. ERG results indicated a significant increase in A-wave amplitude in the 6mo 5xFAD mice and a significant decrease in A-wave amplitude in the 9mo 5xFAD mice. The 5xFAD mice also showed a significant increase in B-wave amplitude at 6mo and a decrease in B-wave amplitude at 9mo. The B-to-A wave ratio indicated dysfunction in the photoreceptors. The 9mo 5xFAD males specifically showed a significant decrease in A-wave amplitude. Investigation of whether Aβ plaques lead to changes in the number of RGC axon terminals showed decreased RGC axon terminals in the 9mo 5xFAD mice following analysis of vGlut2 density in the dLGN. Sholl analysis of 6mo C57 and 5xFAD mice indicate no postsynaptic structural changes. Findings from OMR showed no difference in visual acuity or contrast sensitivity between the C57 and 5xFAD mice.

CONCLUSIONS: Even with histopathological evidence of disease in the brain, there were only modest changes on the visual system structure and function in 5xFAD mice. This suggests that Aβ might have fairly modest influences on the visual system or may point to adaptive mechanisms that preserve function of visual pathways in this model. Investigating how AD pathology affects the early visual system may lay the essential foundation for future studies by delivering insights into how the visual system functions during an AD-like state and may have larger implications as the results of this work could facilitate the development of early diagnostic tools or drug therapies for AD.

Poster #22

Complement Dependent Cytotoxicity Drives Retinal Ganglion Cell Loss in a Novel In Vivo Model of Neuromyelitis Optica

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PURPOSE: Visual impairment from optic neuritis (ON) is a common manifestation in aquaporin-4 (AQP4) autoantibody seropositive neuromyelitis optica spectrum disease (NMOSD). The purpose of our study was to determine whether human recombinant AQP4 antibodies (AQP4 rAb) with different antibody effector function elicits distinct patterns of optic nerve injury in an in vivo model of NMOSD ON.

METHODS: Long Evans rats (n=10/group) received a unilateral subarachnoid injection of 100 ng of anti-AQP4 recombinant antibody (AQP4 rAb) or isotype control (2B4) underneath the optic nerve sheath. AQP4 rAb contained either an intact human IgG1 Fc region (wt) or Fc region mutations that enhanced or diminish complement dependent (CDC) or antibody-dependent cellular (ADCC) cytotoxicity. Impairment in visual acuity and changes in retinal nerve fiber layer (RNFL) and retinal ganglion cell (RGC) complex thickness were determined 28 days later using optokinetic tracking response and optical coherence tomography. Statistical analyses were performed using ANOVA with Tukey's test.

RESULTS: Injection of wt AQP4 rAb cause a decline in visual acuity when compared to rats having received isotype control 2B4 rAb (wt AQP4=0.34±0.03c/d vs. 2B4= 0.4±0.07c/d; p=0.02). Visual impairment was also observed in rats after injection of AQP4 rAb with enhanced CDC (CDC++/ADCC- AQP4=0.34±0.03c/d, p=0.001). When compared to baseline values recorded prior to injection, significant RNFL thinning was most evident in rats that have received either wt AQP4 or AQP4 rAb with enhanced CDC and ADCC (percentage RNFL loss in wt AQP4=-10±3%, p=0.002; CDC+++/ADCC+ AQP4=-9±5%, p=0.023). Mutated AQP4 rAb without ADCC function (CDC++/ADCC-) showed intermediate loss (-6±3%, p=0.32). No RNFL thickness loss was noticed in animals having received 2B4 (-1±5%) or mutated AQP4 rAb with ablated CDC (CDC-/ADCC++= 0±3%). Analysis of the RGC complex revealed significant thinning in rats injected with wt AQP4 (77±3 µm, p=0.003), CDC++/ADCC- AQP4 (76±4 µm, p=0.003), and CDC+++/ADCC+ AQP4 (76±4 µm, p=0.003).

CONCLUSIONS: Similar to models of intracerebral NMOSD pathology, NMOSD optic nerve injury is dependent on targeting the AQP4 water channel through antibody-mediated CDC. Magnitude of visual impairment and RGC degeneration were differentially impacted by ADCC.

Poster #24

Visualization and Quantification of the Spatial Patterns of Edema and Atrophy in Non-Arteritic Anterior Ischemic Optic Neuropathy (NAION) Over Time Based on Deep-Learning Variational-Autoencoder

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PURPOSE: Acute NAION causes optic nerve head (ONH) swelling and spatial distortion changing over time and have not been well characterized. We hypothesize a deep-learning bi-channel variational autoencoder (VAE) can be used to create a latent space that reflects the spatial patterns of the ONH that changes as the pathological process evolves.

METHODS: We performed VAE training on the optical coherence tomography (OCT) ONH scans over time from 322 NAION subjects (1524 scans; Quark study) and 97 normal subjects (797 scans). The trained VAE created latent spaces of the retinal nerve fiber layer (RNFL) and total retinal (TR) thickness maps that depicted the continuum of spatial patterns and can monitor each NAION eye progressing over time in relation to the normal and all NAION eyes. Each latent space, consisted of a montage of 21 x 21 color thickness map of RNFL or TR, whose spatial patterns were encoded by only two latent variables, one on the x-axis and the other on the y-axis of the map.

RESULTS: The VAE latent space montage maps display meaningful spatial patterns of edema and atrophy at the ONH that could be visually observed and located in different regions in the latent space map for individual eyes. In our independent test dataset (15 NAION and 15 normal subjects), the Pearson's correlation coefficient of the mean peripapillary thicknesses between the input and reconstructed images was higher than 0.98 ($p < 0.001$) through each RNFL or TR VAE channel.

CONCLUSIONS: The trained VAE successfully described and quantified essential spatial information of the pattern of edema and eventual atrophy using only two latent variables. Latent space montage maps can classify different patterns and severity of swelling/atrophy in NAION. The quantification and characterization of the spatial patterns simplify the structural measurements that can be related to vision loss at presentation and outcome.

Poster #1

Umbilical amnion for posterior lamellar eyelid reconstruction and anopthalmic socket reconstruction: a retrospective chart review

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PURPOSE: Traditionally, autogenous grafts such as oral mucous membrane, hard palate, and dermis fat grafts have been used for posterior lamellar and anophthalmic socket reconstruction. Due to donor site morbidity, other materials, such as umbilical amnion, have been studied in small case studies for anophthalmic socket reconstruction and cicatricial entropion repair. However, its use in primary eyelid reconstruction has not been described.

METHODS: An IRB-approved retrospective review was performed to identify patients undergoing anophthalmic socket and eyelid reconstruction with umbilical amnion at a single, academic tertiary care institution between June 2019 and March 2023. Data collected included: patient age; indication for surgery; length of post-operative follow-up; and outcome measures of eyelid position, surgical site infection, wound dehiscence, reoperation, and cosmetic result. Surgeries were considered successful if the socket retained a prosthesis; the corneal epithelium was stable or improved post-operatively; the cosmetic appearance was favorable; and there was no post-operative eyelid malposition, retraction, epiphora, or reoperation.

RESULTS: Twenty-two patients with a mean age of 53 and an average follow-up of 10 months received umbilical amnion. Thirteen anophthalmic socket reconstructions were performed on 13 sockets in 12 patients. Reoperation was not required in 10 sockets and 5 sockets had proper eyelid position. Complications included: 1 surgical site infection, 1 dehiscence, 8 mispositioned eyelids, and reoperation on 3 sockets. Umbilical amnion was used in 10 sighted patients for 3 primary posterior lamellar eyelid reconstructions after Mohs surgery, 1 medial canthus reconstruction after lesion excision, 3 palpebral conjunctival reconstructions, 1 symblepharon repair, and 2 cicatricial eyelid retractions. Of these patients, 1 patient was lost to follow-up. There were no surgical site infections, 1 wound dehiscence, 2 mispositioned eyelids, and 2 reoperations.

CONCLUSIONS: Umbilical amnion can be effective for anophthalmic socket contracture reconstruction and provides successful outcomes in patients undergoing primary posterior lamellar eyelid reconstruction. However, due to the nature of injuries seen in some patients with anophthalmic sockets, healing can be difficult and multiple surgeries may be necessary. Nonetheless, umbilical amnion is well tolerated, eliminates

donor site morbidity, and reduces surgical times. Further studies are needed to compare the effectiveness of umbilical amnion to other grafts in primary posterior lamellar reconstruction of the eyelid.

Poster #3

Inhibition of HIF-1 Signaling Reduces Visual Impairment in a Mouse Model of Multiple Sclerosis-like Optic Neuritis

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PURPOSE: Optic neuritis (ON) is often an early sign of multiple sclerosis (MS) and recent studies show a link between HIF-1 pathway activation and inflammation. This study aimed to determine if inhibition of the HIF-1 pathway using the HIF-1α antagonist Acriflavine (ACF) can reduce clinical progression and rescue the ocular phenotype in an experimental autoimmune encephalomyelitis (EAE) ON model.

METHODS: EAE-related ON was induced in 40 female C57BL/6J mice by immunization with MOG33-55, Complete Freund's Adjuvant and pertussis toxin. Twenty EAE mice received daily systemic injections of ACF at 5 mg/kg, 20 EAE mice were placebo injected and another 20 naive mice served as controls. Clinical progression was monitored using a 5-point EAE scoring scheme. Visual acuity was assessed weekly and RGC complex thickness was measured using optical coherence tomography (OCT). Retinal Ganglion Cells (RGC) and optic nerve axons were counted using RBPMS and PPD staining. Grades of demyelination (0-3 grading) and numbers of CD3+ cells in optic nerves were evaluated by H&E/LFB and immunohistochemistry. Differences were analyzed by ANOVA and Tukey's post-hoc test.

RESULTS: ACF-treated EAE animals show significantly less motor-sensory impairment (Area under curve EAE:51±3 vs. EAE+ACF: 28±3; p=0.0001). ACF treatment also preserved visual acuity (EAE:0.24±0.06 c/d vs. EAE+ACF: 0.290.006 c/d; p=0.01) and maintained RGC complex thickness when compared to untreated EAE mice (EAE:61±3µm vs. EAE+ACF:64±3µm; p=0.003). Higher RGC density (EAE:248±19 RBPMS cells/field vs. EAE+ACF: 275±16 RBPMS cells/field; p=0.02) and axon numbers (EAE:1310±562 axons/field vs. EAE+ACF: 2096±527; p=0.008) were observed in ACF treated EAE mice when compared to EAE animals in the placebo group. Grade of optic nerve demyelination was lessened (EAE:2.1±0.5 vs. EAE+ACF:0.9±0.9; p=0.047) and less infiltrating CD3+ cells were observed (EAE:98±32 cell/mm vs. EAE+ACF: 31±20 cells/mm; p=0.0001) in ACF treated EAE mice.

CONCLUSIONS: In EAE mice, daily ACF injections reduce motor-sensory impairments as well as increase visual system recovery, including preservation of the RGC density and optic nerve axon number. In conclusion, these improvements seem to be attributed to the inhibition of the HIF-1 pathway by ACF. Further experiments are needed to determine ACF's mechanisms of action in more detail but is a promising approach for MS therapies.

Poster #5

Utilization of Ketogenic Diet Worsens Visual and Motor-Sensory Deficits in An Animal Model of Multiple Sclerosis

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PURPOSE: Controversy exists regarding the benefits of ketogenic diet (KD) utilization as adjuvant therapy in multiple sclerosis (MS). The purpose of this study was to establish the primary effects of KD on visual function and structure in an experimental autoimmune encephalomyelitis (EAE) mouse model of MS.

METHODS: EAE was induced in the 80 female C57BL/6J by immunization with MOG33-55, complete Freund's Adjuvant, and pertussis toxin while another 16 mice served as a naïve control group. The 80 EAE induced mice were assigned into cohorts (n=20/group) to stay on the standard rodent chow (EAE) or to start KD either 2 weeks before EAE induction (preconditioning), at EAE induction (prophylactic), or at the onset of symptoms (late intervention). Mice were scored daily for motor-sensory impairments using a mobility scale (0=normal to 5=death). Visual acuity was assessed using optokinetic responses (OKR). Visual structure was assessed by measuring the retinal nerve fiber layer (RNFL) thickness. Pattern electroretinography (pERG) and visual evoked potentials (VEP) were recorded at the end of the experiment. Tissue from the retinas, optic nerves, brain, and spinal cord were sectioned for subsequent analysis with microscopy. All data were analyzed using one- and two- way ANOVA followed by post hoc tests.

RESULTS: EAE animals from the preconditioned KD group showed significantly worse motor-sensory impairment relative to EAE controls (Area under curve EAE score: EAE: 58±2, pre KD: 68±3, $p<0.001$; pro KD: 60±3, $p=0.09$; late KD: 58±3). Similarly, visual acuity data showed worse OKR tracking in the preconditioned KD group (0.23±0.05 cycles/degree) compared to naïve (0.38±0.03 cycles/degree, $p<0.0001$) and EAE controls (0.26±0.05 cycles/degree; $p=0.024$). There was no significant difference between the standard diet EAE controls and the pro KD (0.24±0.06 cycles/degree) and late KD group (0.25±0.05 cycles/degree), but all three EAE groups had significantly lower visual acuity and EAE scores than the naïve group ($p<0.0001$). Compared to naïve mice, average RNFL thickness decreased significantly in all EAE induced mice (naïve: 69±2μm vs. EAE: 66±4μm; $p=0.001$, pre KD: 66±4μm; $p=0.002$, pro KD: 67±4μm; $p=0.02$, late KD: 66±3μm; $p=0.001$) whereas differences between these EAE induced groups were not significant.

CONCLUSIONS: This study identified that implementing KD negatively influenced motor-sensory and visual function, and that preconditioning the mice with KD before EAE induction resulted in the worst structural and functional outcome. These data suggest that KD should not be recommended for patients with MS.

Poster #7

In vivo investigation of mia2/mia3 in protein transport within zebrafish photoreceptors

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PURPOSE: From a mutational screen, we identified two related factors essential for maintaining age-related photoreceptor health in zebrafish, Mia2 and Mia3. Of significance, mutations in human and dog MIA3 have very recently been shown cause retinopathies. How the proteins Mia2 and Mia3, whose known role is assisting protein transport, affect photoreceptor health is uncharacterized. MIA2/MIA3 have been described to act at ER exit sites to assist large protein transport from the ER to Golgi. In addition to large protein transport, unconventional protein trafficking that circumvents the Golgi occurs in photoreceptors through poorly defined mechanism(s). We hypothesize Mia2/Mia3 serve unique functions within photoreceptors to accommodate trafficking of large proteins, but also functions in the unique unconventional trafficking pathway. To test this hypothesis, Mia2/Mia3 will be investigated through gene manipulation followed by detailed phenotype analysis.

METHODS: mia2 and mia3 mutants were created using Crispr/Cas9 genomic deletions. Transgenic Markers 5XATF6RE:d2GFP and gnat2:Lc3-eGFP-mcherry were used to quantify ER stress and autophagy respectively. Electron microscopy was utilized to characterize photoreceptor ultrastructure and OCT was used to detect altered photoreceptor morphology within mia2/ mia3 Mutants.

RESULTS: Zebrafish mia2 mutants showed neuronal defects including photoreceptor degeneration. Consistent with problems with protein transport mia2 mutants showed increased ER Stress. Electron microscopy revealed defects in photoreceptor ultrastructure, presenting with ER vesiculation, autolysosome vacuolation, and stunted outer segments. Additionally, OCT imaging of mia2 mutants revealed a reduction of photoreceptors that decreased further as the fish aged into adulthood. Interestingly and in contrast to mia2, mia3 mutants alone did not display any photoreceptor defects. However, double mia2/mia3 mutants showed exacerbated phenotypes of mia2 mutants.

CONCLUSIONS: mia2 mutants display photoreceptor defects while mia3 mutants do not show photoreceptor defects. During the completion of this research a new isoform of mia3; Mia3s was described. The mutation created for mia3 currently only affects the Mia3l isoform. Research is underway to generate mia3 mutants which knock out Mia3s and both Mia3s and the Mia3l concurrently. In addition to detailed phenotype analyses on these newly generated mutants, we will identify binding partners of Mia2/3s/3l through in vivo proximity proteomics.

Poster #9

Labeling and enrichment of retinal proteins using non-canonical amino acids

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PURPOSE: Understanding the spatial and temporal expression pattern of different proteins in each of the ~100 retinal cell types provides essential information about the dynamic regulation of diverse processes in the retina under normal and diseased conditions. However, analysis of the retinal proteome is limited by the inability to apply a single type of chemistry to label proteins for subsequent purification and analysis. Bio-Orthogonal Non-Canonical Amino acid Tagging (BONCAT) may provide a solution to that problem. The goal of this project is to test the efficiency of applying BONCAT to the mouse retina.

METHODS: Azidohomoalanine (AHA) is a non-canonical amino acid with an azide functional group that is used as a methionine substitute. AHA, or PBS as the negative control, was delivered to C57Bl6/J mice (male and female; age 1-6 months) by intraperitoneal injection. Mice were fed either normal chow or a low methionine diet. A range of AHA doses (2-10 mg per 20 g body weight) were delivered for 1-4 days. Subsequently, retinas were harvested and stored at -80C. Proteins from stored retinas were extracted using a lysis buffer containing a broad-spectrum protease and phosphatase inhibitor cocktail (Roche). AHA containing proteins were labeled using a copper-dependent click reaction (Click-&-Go Protein Reaction buffer kit, Click Chemistry Tools) with alkyne-biotin. Proteins were methanol-chloroform precipitated prior to analysis by western blotting, with or without concentration on neutravidin beads. Retina function was tested using ERG.

RESULTS: Western blotting using a streptavidin antibody demonstrated labeling of biotin-clicked retina proteins in AHA injected mice with low non-specific labeling in PBS injected mice. Placing mice on a low methionine diet throughout the experiment increased the amount of labeled protein. An AHA dose of 2 mg per 20 g body weight is sufficient. Overall labeling intensity increased with the number of daily AHA injections. This dose did not cause acute retina toxicity since there was no change in ERG.

CONCLUSIONS: Systemically delivered AHA is robustly incorporated into mouse retina proteins. Click chemistry can be used to covalently attach biotin tags for subsequent proteomic analysis.

Poster #11

Corneal sensitivity in chronic orbital pain treated with peripheral trigeminal nerve blocks

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PURPOSE: To determine corneal sensitivity in chronic orbital pain patients, risk for corneal hypoesthesia associated with peripheral trigeminal nerve blocks, and whether corneal sensitivity correlates with the subjective complaints of orbital pain patients.

METHODS: Chronic orbital pain patients treated with unilateral peripheral trigeminal nerve blocks and controls were enrolled in the IRB-approved prospective trial. Pain subjects completed Cochet-Bonnet (CB) aesthesiometer evaluations prior to injection, 30 minutes post-injection, and at 2-4 weeks follow-up. Controls underwent CB evaluations once. All subjects completed the Headache Impact Test (HIT-6), the Leeds Assessment of Neuropathic Symptoms and Signs (LANSS), and the Utah Photophobia Symptom Impact Scale (UPSIS-12) for subjective evaluations of their headache, neuropathic pain, and photophobia.

RESULTS: Mean baseline corneal sensitivity thresholds of the injection and fellow eyes of 6 orbital pain patients were 5.75 ± 0.42 cm and 5.83 ± 0.41 cm, respectively, with no significant inter-eye asymmetry (p=0.7). The mean sensitivity thresholds for the 11 controls were 5.95 ± 0.11 cm (OD) and 5.94 ± 0.15 cm (OS) (p=0.8). The baseline thresholds for pain patients were not significantly different from those of controls (p=0.3). The mean sensitivity threshold of the injection eyes was 5.78 ± 0.25 cm at 30 minutes post-injection (p=0.9) and 5.92 ± 0.20 cm at follow-up (p=0.5), similar to baseline. A higher neuropathic pain score was significantly correlated with lower corneal sensitivity after adjusting for age when evaluating all subjects (R = -0.7, p = 0.005), but the correlation was not significant when evaluating the pain group alone (R = -0.8, p = 0.1). Headache and photophobia symptoms did not correlate significantly with corneal sensitivity.

CONCLUSIONS: Chronic orbital pain patients do not display corneal hyperesthesia. Peripheral trigeminal nerve blocks do not alter corneal sensitivity. Neuropathic symptoms reflective of trigeminal neurocutaneous hypersensitivity may correlate with corneal hypoesthesia.

Poster #13

Methylenetetrahydrofolate reductase expression in human diabetic retinas

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PURPOSE: Methylenetetrahydrofolate reductase (MTHFR) is a key enzyme responsible for remethylating folate, a vital cofactor necessary for converting homocysteine to methionine. When the MTHFR gene is mutated, homocysteine, a non-proteinogenic amino acid, begins to accumulate. Increased homocysteine levels have been associated with oxidative stress. These high levels have been linked to several retinal diseases, such as diabetic retinopathy. Previously, it has been found that MTHFR expression is associated with the function of retinal pigment epithelium. The health of the retina is largely dependent on the role of folate. When that pathway is disturbed, the proper function of the retina is at risk. Research quantifying the expression of MTHFR in human eyes with diabetic retinopathy has not been studied. This study aims to assess the retinal expression of MTHFR protein in Lewis rat controls, normal human eyes, and human diabetic retinas.

METHODS: Analysis of MTHFR protein expression was performed using immunohistochemical analysis (IHC). Samples included a Lewis rat eye as a control, and human eye samples from National Disease Research Interchange. The human eye samples were from both normal and diabetic retina cross sections. These sections were stained with MTHFR and PDGFR-β primary antibodies. This was followed by labeling with secondary antibodies anti-mouse for PDGFR-β and anti-rabbit for MTHFR. Primary antibody was not added to the rat control sample. DAPI was used as a nuclear stain and mounting medium. Analysis to quantify fluorescence intensity was measured using ImageJ software. Background signal was accounted for by using the equation for corrected total cell fluorescence.

RESULTS: IHC analysis revealed robust expression of MTHFR staining throughout the retina with higher expression in the photoreceptor outer layer in both rat and human retinas. The Lewis rat control image revealed a value of 58 a.u. In comparison, the normal human eye sample had 266 a.u, whereas the human diabetic retina showed 86 a.u. After quantifying the intensity of fluorescence of MTHFR throughout the retina, the data revealed a decreased intensity in the diabetic retina.

CONCLUSIONS: Diabetic retinopathy is the leading cause of blindness in working-aged adults. The importance of identifying pathways that contribute to the progression of the disease is vital. Knowing that MTHFR plays a role in the functional health of the retina allows for a multifactorial role in understanding diabetic retinopathy. This study revealed that MTHFR protein expression is decreased in human eyes with diabetic retinopathy compared to MTHFR protein expression in normal human eyes.

Poster #15

Potential Treatment of Elevated Homocysteine on Retinal Pigmented Epithelial Cells

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PURPOSE: Homocysteine is a sulfur containing non-proteinogenic amino acid involved in methionine metabolism. Elevated homocysteine levels is known as hyperhomocysteinemia (Hhcy) and is implicated in several ocular disorders such as glaucoma, diabetic retinopathy, and age related macular degeneration (AMD). Retinal pigmented cells (RPE) are the primary cellular target for pathologic changes in AMD. Hhcy is known to induce reactive oxygen species production, which can cause direct injury to proteins, lipids, and nucleic acids- leading to cell death. Melatonin is a hormone important for circadian rhythms, but is also an antioxidant. Limited data in the literature exists regarding the role of melatonin as a potential treatment for Hhcy. The purpose of the current study was to evaluate the therapeutic potential of melatonin in Hhcy using the RPE cell culture model system.

METHODS: ARPE-19 cells were grown in DMEM F-12 media supplemented with 10% FBS and 1% Penicillin/Streptomycin. MTT assay, Apoptosis/Necrosis Assay, and oxidative stress assays were used to determine manor of cell death of ARPE-19 cells after treatment with homocysteine (1, 5, 10, 50, 100, 500, 1000 μM) and melatonin (5 μM). Changes in cellular morphology were determined by confocal scanning microscope images taken pre-treatment, 24, 48, and 72 hours post treatment. Hydrogen Peroxide acted as the positive control for cellular death. Data was analyzed using a one-way ANOVA. Statistics were analyzed using the GraphPad Prism 9 system.

RESULTS: As concentrations of homocysteine increased, instances of ARPE-19 cell viability decreased with significant values ($p<0.001$) at 50, 500, and 1000 μM homocysteine. Cells treated with melatonin or co-treated with homocysteine and melatonin resulted in increased cell viability. Cells treated with homocysteine resulted in cellular death via apoptosis, however, cells co-treated with melatonin showed a decrease in cellular death via apoptosis. Cells treated with melatonin showed a decrease in green fluorescence- corresponding to a decrease in oxidative stress, when compared to the positive control. Abnormal cellular morphology was observed in cells treated with homocysteine at concentrations over 50 μM, and conditions worsened at longer treatment duration.

CONCLUSIONS: By treating ARPE-19 cells with homocysteine, we found that homocysteine leads to cell death via apoptosis at concentrations above 50 μM. We also found that APRE-19 cells co-treated with homocysteine and melatonin resulted in an increase in cell proliferation, and that melatonin shows protective effects against

oxidative stress. This demonstrates that melatonin has potential protective effects against elevated homocysteine.

Poster #17

Increased T-regulatory cell activity protects retinal ganglion cells in glaucoma

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PURPOSE: Our previous studies have shown that experimental glaucoma in mice activates T cell-driven autoimmune response that can further contribute to vision loss. Regulatory T cells (Tregs) are a specialized subset of T cells that suppress immune responses, thereby maintaining homeostasis and self-tolerance. Here, we sought to explore whether induction of increased T-regulatory cell (T-reg) activity ameliorates vision loss in mice with glaucoma.

METHODS: Lymphocyte activation gene 3 (Lag3), also known as CD223, negatively regulates T cell activation, proliferation, and cytokine production. We induced ocular hypertension (OHT) in Lag3fl/fl.Foxp3CreERT2-GFP mice to establish a chronic glaucoma model. Intraperitoneal injection of tamoxifen was used to conditionally remove the Lag3 gene Treg cells of adult mice to increase their abundance and activity. Mice were divided into 5 groups: naïve (n=10), tamoxifen without OHT (n=7), Untreated (OHT without tamoxifen, n=8), Prevention (tamoxifen before OHT, n=9) and Treatment (tamoxifen after OHT, n=10). Visual function was assessed monthly by optokinetic response testing. 12 weeks after OHT induction, optic nerve damage was determined by p-phenylenediamine (PPD) staining.

RESULTS: Tamoxifen injection increased the proportion of CD4+ Treg cells detected in peripheral lymph nodes (naïve vs tamoxifen injected, 14.6%vs18.8%, p=0.0287) and the spleen (naïve vs tamoxifen injected, 10.8%vs15.5%, p=0.0043). 12 weeks after induction of OHT, mice in the Treatment group showed higher visual function when compared to those in the Untreated group (0.366 c/d vs. 0.322 c/d, p=0.027). However, mice in the Prevention group did not perform significantly better than those in the Untreated group (0.336 c/d, p=0.89). Histologically, significantly fewer damaged axons were observed in the optic nerves of the Treatment group when compared to the Untreated group (9.1±6.2 vs. 20.4±14.2, p=0.036). Animals in the Prevention group displayed slightly less damage than untreated mice, but statistical significance was not reached (13.4±5.4, p=0.3532).

CONCLUSIONS: These results demonstrate that increasing Treg cell activity can reduce experimental glaucoma damage in mice. Our findings suggest that immunomodulatory therapy aimed to minimize the impact of T-cell mediated damage in glaucoma may be beneficial for some patients.

Poster #19

A Dose Response Study for Subretinal Gene Therapy for Treating X-Linked Juvenile Retinoschisis in a Retinoschisin 1 Knockout Mouse Model

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PURPOSE: X-linked Retinoschisis (XLRS) causes macular degeneration due to loss-of-function of the retinoschisin 1 (RS1) gene, causing reduced visual acuity. A human clinical trial for treating XLRS showed that high-dose intravitreal gene therapy led to ocular inflammation without phenotype improvement. Subretinal gene delivery's superiority over intravitreal approach for treating RS1 remains unclear. Therefore, we utilized a novel adeno-associated viral vector (AAV) for subretinal RS1 gene delivery in a pre-clinical Rs1-KO mouse model. This AAV2tYF vector contains three tyrosine to phenylalanine (Y-to-F) mutations which have been shown to improve its transduction efficiency. This vector is FDA-approved for clinical applications. To determine the optimal dose range for treating XLRS via subretinal delivery, we conducted a dose-response study using this vector to deliver the RS1 gene in mice. Long-term effects of retinal rescue after rAAV2tYF-CB-hRS1 subretinal gene delivery were investigated.

METHODS: Rs1-KO (Rs1-/y and Rs1-/-) mice were subretinally injected with 2 µL of rAAV2tYF-CB-hRS1 vector with different doses at postnatal days 24-31. Rs1-KO mice were injected with different doses, receiving either the highest dose of 6E9 viral genomes (vg)/eye (n=5), 6E8 vg/eye (n=5), 6E7 vg/eye (n=5), or injection of the diluent alone as a sham injection (Alcon BSS + 0.014% (v/v) Tween buffer), (n=6) and 22 untreated eyes. Analysis of retinal function by electroretinography (ERG) and structural analysis by measuring cyst severity and outer nuclear layer thickness using optical coherence tomography (OCT) were performed on all mice 1 to 3 months post (MP) injection. Functional vision of treated mice was evaluated using a visually guided swim assay. Rod- and cone-dependent visual pathways as measured by ERG and severity of retinal cysts of vector-treated mice were compared to those of diluent-treated mice (sham control group) and completely untreated mice (treatment naïve group).

RESULTS: At 1 MP treatment, treated Rs1-KO eyes with the 6E8 vg/eye dose had a strong response to the 3.0 cd·sec/m2 bright flash, with higher b-wave values and showed a significant improvement in the amplitudes of the 5-Hz flicker ERG compared to both sham-treated and untreated eyes. This improvement was also observed at 2 and 3 MP treatment. These findings show that subretinal gene delivery was able to reestablish the electrical function in the cone photoreceptor cells in ERG. High and low-dose groups did not improve retinal function in rods or cones pathways, despite reduced cyst severity scores. At all doses, cyst severity scores were significantly reduced compared to the untreated eyes but not to the sham-injected eyes. These observations

indicate that the ability to rescue retinal function by gene augmentation in XLRS is highly dose-dependent and that the observed improvements in ERG are not entirely accounted for by the amelioration of cysts in this disease.

CONCLUSIONS: In conclusion, we conducted a dose-response study to determine the optimal dosing for treating RS1 using subretinal gene therapy. Based on the results we concluded that subretinal gene therapy with a dose of 6E8 vg/eye improves retinal function and structure by sustaining photoreceptor cell viability in treated eyes, restoring the electrical function of cone photoreceptors, and reducing cyst severity scores. However, Cyst severity score results indicate that even a low amount of RS1 protein production in the eye promotes the resolution of schisis cysts. Even though the presence of a low amount of RS1 protein appears to ameliorate schisis in the retina, the rescue of retinal electrical function requires a higher gene dose. The findings in this study provide a vector dose and an approach that facilitates the clinical translation of this therapy and can be extended to human trials.

Poster #21

Automated Optic Disc Finder and Segmentation Using Deep Learning for Blood Flow Studies in the Eye

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PURPOSE: In-vivo imaging with laser speckle flowgraphy (LSFG) enables optical measurement of an index of blood flow in the retina. LSFG can help observe blood flow changes in various diseases, including optic nerve problems such as glaucoma, ischemic optic neuropathy, and others. The purpose of this study is to develop an automated method for the identification of the optic disc in the LSFG images. Automated identification of the optic disc in LSFG images is particularly challenging because of limited contour information in the rendered blood flow maps. In this study, we adopted a state-of-the-art U-Net approach (nnU-Net) to automatically segment the optic disc region based on the paired LSFG blood flow image and infrared light intensity map.

METHODS: One hundred subjects (training/test dataset ratio: 70/30) were used in this study. A trained neuro-ophthalmologist (Expert 1) traced the optic discs in color fundus photographs from the same eye, and then these masks were registered into the LSFG domain. The nnU-Net was trained to identify the optic disc just based on the LSFG blood flow composite and infrared light intensity images. After training, we compared the difference between nnU-Net's output and Expert 1 with the difference between Expert 1 and a second clinician (Expert 2) in the test dataset.

RESULTS: The mean (and standard deviation) Dice coefficient was 0.92 ± 0.04 between the nnU-Net and Expert 1’s tracing and 0.89 ± 0.06 between Expert 2’s and Expert 1’s tracings, respectively. The mean intersection over union (IoU) index was 0.86 ± 0.06 between the nnU-Net and Expert 1’s tracing and 0.81 ± 0.09 between Expert 2’s and Expert 1’s tracings. Both evaluation metrics showed the nnU-Net's predictions were significantly closer to Expect 1's tracing than Export 2's (p-values < 0.001).

CONCLUSIONS: This study demonstrated that the nnU-Net’s predictions for optic disc segmentation were significantly closer to Expert 1’s manual tracings compared to Expert 2’s tracings. In summary, having a robust optic disc segmentation in LSFG can reduce tedious manual tracing and will also be a foundation for future developments of automated region-based feature extraction in LSFG and potentially for other imaging modalities.

Poster #23

Antioxidant treatment suppresses retinal defects in Drosophila models of SNRNP200-associated Retinitis Pigmentosa

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PURPOSE: Retinitis pigmentosa (RP) is a collection of genetic retinal degeneration disorders that affects 1:4,000 individuals worldwide. RP initially presents with a loss of rod photoreceptors, followed by a loss of cones. The disease is progressive and can result in complete blindness. RP33 is a non-syndromic form of autosomal dominant RP caused by mutations in the SNRNP200 gene encoding a core pre-mRNA splicing factor. The pathological mechanisms of RP are not well understood; however, there is evidence of oxidative stress in the retina. Our goal is to understand how mutations in SNRNP200 causes retinal defects and identify potential treatments.

METHODS: To determine how mutations in SNRNP200 cause retinal defects, we developed Drosophila models possessing mutations identified in humans with RP. Drosophila has an orthologue of SNRNP200 that we refer to as dSnrnp200. Human and Drosophila SNRNP200 exhibit 74% amino acid identity and 89% similarity. CRISPR mutagenesis was used to introduce mutations into Drosophila dSnrnp200. These mutations include a de novo mutation called Bigfoot because it is a private mutation. The Bigfoot mutation causes amino acid substitution T731I. In addition, a mutation that causes S1087L was generated. RNAi was also used to knock-down dSnrnp200 in differentiated cells of larval eye imaginal discs, which give rise to the adult eye. Retinal defects were detected in in dSNRNP200 mutants and upon depletion. To determine if oxidative stress contributed to these defects, we fed flies either N-acetyl cysteine (NAC) or water as a control. NAC is an FDA approved antioxidant.

RESULTS: Flies with the Bigfoot mutation are homozygous lethal. By contrast, flies with the mutation that causes S1087L are homozygous viable. Both dSnrnp200 mutations increase apoptosis in larval eye imaginal discs, relative to controls. The mutant larvae develop into adults that have abnormal electroretinograms, but no other apparent defects. The photoreceptors in these mutants show an abnormal pattern of organization which was partially corrected upon treatment with NAC. Depletion of dSnrnp200 increases apoptosis in larval eye imaginal discs and produced a rough eye phenotype in adults. NAC treatment decreased apoptosis in the larval eye discs and showed a dosage-dependent suppression of the rough eye phenotype.

CONCLUSIONS: Taken together, these data demonstrate that the Drosophila models of SNRNP200-associated RP recapitulate aspects of the human disease. Currently,

NAC is being used in a clinical trial for individuals with RP; however, it is not known if individuals with SNRNP200 mutations benefit. Our findings strongly suggest that individuals with SNRNP200-associated RP will benefit from NAC treatment and that oxidative stress plays a key role in the pathogenesis.

Poster #25

Role of SIN3A dysregulation in neural crest differentiation and Witteveen-Kolk syndrome

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PURPOSE: Childhood-onset glaucoma is commonly a manifestation of multi-organ syndromes, such as Witteveen-Kolk syndrome (WITKOS). WITKOS was defined in 2016 and is due to a loss of function mutation in *SIN3A*, which encodes a histone deacetylase scaffold and is critical to neural development. While ocular dysgenesis is not a common feature of WITKOS, a parent and two children presented to UIHC with early-onset glaucoma and a confirmed *SIN3A* mutation. We aimed to quantify *SIN3A* gene expression at the neural crest stage of anterior segment development. We also aimed to assess the gene's potential role in the establishment of neural crest identity.

METHODS: Urine epithelial cells were collected non-invasively from all three family members and primary cultures were established; *SIN3A* mutations were confirmed by Sanger sequencing. Neural crest-like status was induced in human induced pluripotent stem cells (hiPSC) using a specialized growth factor medium. *SIN3A* gene expression was assessed using real-time quantitative polymerase chain reaction (RT-PCR) in hiPSCs and the induced neural crest cells. Flow cytometry against CD49d and p75/NGFR was used to evaluate neural crest identity.

RESULTS: Primary cell cultures were established from cells collected from the patients' urine samples. *SIN3A* mutations were confirmed in DNA extracted from patient cells. Exposure of hiPSCs to an induction medium resulted in a relative increase in expression of markers of neural development.

CONCLUSIONS: This project evaluates SIN3A expression in induced neural crest cells to characterize anterior segment development in WITKOS. First, we found that collecting cells from patient urine is a viable approach to establishing primary cell lines and therefore allows a non-invasive method to collect human samples. Second, hiPSCs can be differentiated into neural crest using specialized media. These techniques can be used to investigate molecular pathways disrupted by genetic or epigenetic mutations, as well as to model early ocular dysgenesis. Future goals include defining the changes in Sin3a protein levels after translational inhibition and utilizing CRISPR-Cas9 to create loss of function mutations for functional studies. These experiments will characterize the timeline of anterior segment dysgenesis and/or dysregulation in Witteveen-Kolk syndrome and other syndromic causes of childhood glaucoma. In turn, these findings can inform potential gene therapy treatments as well as clinical timepoints to regulate intraocular pressure in pediatric glaucoma.