483 Glaucoma: Biochemical and molecular disease mechanisms
Wednesday, May 10, 2017 3:45 PM–5:30 PM
Exhibit/Poster Hall Poster Session
Program #/Board # Range: 4899–4919/B0317–B0337
Organizing Section: Biochemistry/Molecular Biology

Program Number: 4899 **Poster Board Number:** B0317
Presentation Time: 3:45 PM–5:30 PM
Correlations between cytokine levels in aqueous humor, disease duration and intraocular pressure in acute angle closure glaucoma attack
Tomohito Sato[1], Masaru Takeuchi[1], Yoko Karasawa[1], Takayuki Kanda[1], Masataka Ito[2], Toshio Enoki[3]. [1]Ophthalmology, National Defense Medical College, Tokorozawa, Japan; [2]Developmental Anatomy and Regenerative Biology, National Defense Medical College, Tokorozawa, Japan; [3]ENOKI EYE CLINIC, Sayama, Japan.
Purpose: To identify cytokines in aqueous humor related to disease duration and intraocular pressure (IOP) before and after phacoemulsification in patients with first acute angle closure glaucoma attack.
Methods:
Eleven eyes of 10 patients, in whom phacoemulsification was performed to resolve first acute angle closure glaucoma attack after conservative treatments such as IOP-reducing drops, internal drugs and intravenous drips, were enrolled (males / females ; 4 / 6, average age ± standard deviation ; 73.0 ± 8.64 years old). Undiluted aqueous humor samples were collected at the initiation of the surgery. The levels of VEGF, IL-6, IL-8 and G-CSF in the samples were measured by a multiplex bead array. Correlations between these cytokines and IOP at initial visit (43.7 ± 12.1 mmHg), IOP just before the surgery (33.2 ± 18.8 mmHg), IOP one week later after the surgery (17.0 ± 9.46 mmHg), IOP one month later after the surgery (16.9 ± 6.93 mmHg) and disease duration from awareness to the surgery (11.9 ± 16.3 days) were analyzed using Spearman's correlation. P value less than 0.05 was considered significant.
Results:
There was no significant difference between IOP at initial visit and all kinds of cytokines. Positive relations were shown between IOP just before the surgery and levels of IL-8 and G-CSF. There were negative correlation between IOP at one week after the surgery and level of G-CSF, and between IOP at one month after the surgery and level of IL-8 and G-CSF, respectively. In addition, negative correlations were shown between disease duration and levels of VEGF, IL-8, and G-CSF.
Conclusions:
In eyes with first acute angle closure glaucoma attack, persist high IOP could accelerate cell proliferation and infiltration of inflammatory cells. Elimination of cytokines in the aqueous humor by phacoemulsification would contribute to lowering IOP effect by the surgery.
Commercial Relationships: Tomohito Sato, None; **Masaru Takeuchi**, None; **Yoko Karasawa**, None; **Takayuki Kanda**, None; **Masataka Ito**, None; **Toshio Enoki**, None

Program Number: 4900 **Poster Board Number:** B0318
Presentation Time: 3:45 PM–5:30 PM
Identification and characterization of variants and a novel 4bp deletion in the regulatory region of *SIX6*, a risk factor for Primary Open Angle Glaucoma
MOHD H. SHAH[1], Noemi Tabanera[2, 3], Subbiah R. Krishnadas[4], Manju Pillai[4], Paola Bovolenta[2, 3], Periasamy Sundaresan[1]. [1]Molecular Genetics, Aravind Medical Research Foundation, Madurai, India; [2]Developmental biology, Centro de Biología Molecular Severo Ochoa, CSIC-UAM, Madrid, Spain; [3]CIBERER, ISCIII, Madrid, Spain; [4]Glaucoma Clinic, Aravind Eye Hospital, Madurai, India.
Purpose: The *SIX6* gene has been associated with POAG in different populations. Here, we studied if there exists a genetic association between *SIX6* with POAG and/or its related quantitative endophenotypes in the south Indian population.
Methods: The cohort of individuals analyzed included 265 POAG and 265 age matched controls from the south Indian population. All individuals received a comprehensive ocular examination before the collection of blood samples, to extract genomic DNA. PCR amplification, taqman based allelic discrimination assay, automated sequencing of the *SIX6* and its enhancer region were performed on the purified DNA samples. A novel 4 base pair deletion identified in the *SIX6* enhancer region was functionally tested using transgenesis in zebrafish and *luciferase* assay in *HEK293* cell line
Results: We sequenced the *SIX6* gene and its enhancer region and identified two known rare, two common variants and a novel 4bp deletion in an already characterized putative enhancer region (Chr14:60974427-60974430). In contrast to previous studies, we could not establish a significant genetic association between the rs33912345 (Odds ratio (OR) =0.919, P=0.4) and rs10483727 SNPs at the *SIX1-SIX6* locus OR=0.95, P=0.58) with POAG. However, patients carrying either the rs33912345 'C' or the rs10483727 "T" risk alleles presented a significant and dose-dependent reduction of the peripapillary retinal nerve fiber layer thickness (RNFL), which was more evident in the superior and temporal-inferior quadrants, well in agreement with the knowledge that RNFL become thinner in glaucoma patients. We also established that patients carrying two copies of the ''C'' or ''T'' risk allele presented a statistically significant increase of the vertical cup disc ratio (p=0.012; p=0.009). Using transgenesis in zebrafish and *luciferase* assay, we determined that the newly identified 4bp deletion in the enhancer region significantly reduces reporter expression in retinal ganglion and amacrine cell layers, where human *SIX6* is expressed, suggesting that *SIX6* activity might be reduced in the retina of patients carrying this deletion.
Conclusions: Our data provides additional support to the previously proposed implication of *SIX6* variants as POAG risk factors and further suggests that the reduced levels of *SIX6* expression might be implicated in POAG pathogenesis.
Commercial Relationships: MOHD H. SHAH, None; **Noemi Tabanera**, None; **Subbiah R. Krishnadas**, None; **Manju Pillai**, None; **Paola Bovolenta**, None; **Periasamy Sundaresan**, None

Program Number: 4901 **Poster Board Number:** B0319
Presentation Time: 3:45 PM–5:30 PM
Aqueous humor biomarker of oxidative damage and antioxidant status: Correlations with oxygen and racial differences
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Purpose: Our previous studies identified significantly higher levels of oxygen (pO_2) in anterior segments of patients with prior vitrectomy/cataract surgeries as well as patients of African American background. This prospective, cross-sectional study explores correlations of a biomarker of oxidative damage and antioxidant status in aqueous humor (AH) of human subjects as well as experimental monkey eyes.
Methods: Patients undergoing glaucoma and/or cataract surgery (n=219) were consented and recruited. Intraocular pO_2 measurements were taken prior to surgery with a fiberoptic probe (Oxford Optronix, UK) at five locations in the anterior segment. AH was collected and stored in liquid nitrogen container. Ascorbic acid (AsA) and total reactive antioxidant potential (TRAP) were measured and 8-hydroxy-2'-deoxyguanosine (8-OHdG), a biomarker of oxidative damage, was detected by ELISA (Oxiselect™). Five rhesus monkeys underwent pars plana vitrectomy followed by lens extraction over two-year period. AsA, TRAP and 8-OHdG were measured as in human subjects. T-test/ANOVA and multivariate regression were used for statistical analysis.
Results: Human AH showed AsA and TRAP levels that were significantly lower and pO_2 higher in groups with previous vitrectomy and vitrectomy plus cataract extraction ($p<0.05$, 0.01 respectively) compared to cataract extraction only (1.4±0.6 mM, 479.3±146.7 Trolox units) with no differences between African American vs. Caucasians. Monkeys showed the same results in AsA, TRAP and pO_2 as humans. However, 8-OHdG was significantly increased after vitrectomy ($p=0.03$) followed by lens extraction ($p=0.04$). Preliminary data of human aqueous indicated a significant increase in African American (n=11) compared to Caucasians (n=17;$p=0.015$). Additional human specimen collection and analysis is ongoing with further subgroup analysis.
Conclusions: Decreased antioxidant levels in human subject and monkey AH following vitrectomy and lensectomy may correlate with increased pO_2. Increased 8-OHdG in monkey AH may provide direct evidence of oxidative damage in structures surrounding the anterior chamber specifically trabecular meshwork. Also, increased 8-OHdG AH levels in African Americans may serve as a biomarker and aid our understanding this population's higher risk and severity of open angle glaucoma.
Commercial Relationships: Carla J. Siegfried, None; **Ying-Bo Shui**, None; **Ying Liu**, None; **Andrew J. Huang**, None; **Paul L. Kaufman**, None
Support: NIH Grant EY021515, NEI P30-EY02687, NEI P30-EY016665, Unrestricted grant from Research to Prevent Blindness

Program Number: 4902 **Poster Board Number:** B0320
Presentation Time: 3:45 PM–5:30 PM
Dysregulated expression of POMP and TMEM136 may contribute to impaired proteasome function and endothelial dysfunction in eyes with pseudoexfoliation syndrome/glaucoma
Matthias Zenkel[1], Ursula Schlotzer-Schrehardt[1], Daniel Berner[1], Friedrich E. Kruse[1], André Reis[2], Chiea Chuen Khor[4], Tin Aung[3], Francesca Pasutto[2]. [1]Department of Ophthalmology, University Erlangen Nuernberg, Erlangen, Germany; [2]Institute of Human Genetics, University Erlangen Nuernberg, Erlangen, Germany; [3]Singapore National Eye Center, Singapore, Singapore; [4]Genome Institute of Singapore, Singapore, Singapore.
Purpose: Recently, a genomewide association study based on a large, international sample collection identified new susceptibility loci for pseudoexfoliation (PEX) syndrome/glaucoma (Aung et *al.*, submitted). To determine the pathophysiological role of the three most significantly associated loci (13q12, 11q23.3, 6p21), we investigated the expression and localization of six associated genes in ocular tissues of PEX and control patients.
Methods: Ocular tissues from 21 donor eyes with PEX and 41 age-matched normal eyes without any known ocular disease (control) were analyzed by qRT-PCR for the expression of fms-related tyrosine kinase 1 (FLT1), proteasome maturation protein (POMP), solute carrier family 46 member 3 (SLC46A3) - [13q12: rs7329408], transmembrane protein 136 (TMEM136), Rho guanine nucleotide exchange factor 12 (ARHGEF12) – [11q23.3: rs11827818], and 1-acylglycerol-3-phosphate O-acyltransferase 1 (AGPAT1) – [6p21: rs3130283]. Protein expression levels were analyzed by immunohistochemical and Western blot analyses in 10 PEX and 10 control eyes using antibodies against the 6 genes of interest and lysyl oxidase-like 1 (LOXL1). Tissues were genotyped by TaqMan assays or direct sequencing.
Results: All 6 genes displayed moderate mRNA expression in all ocular tissues analyzed, with highest levels in iris, ciliary body, and retina. For POMP, there was a trend towards reduced expression in the presence of the risk allele of rs7329408. Both mRNA and protein expression of POMP and TMEM136 were significantly reduced by up to 45% ($p<0.005$) in anterior segment tissues of PEX eyes compared to controls. Immunofluorescence analysis showed POMP, a proteasome maturation protein, to be ubiquitously expressed in most ocular cell types; TMEM136, a transmembrane protein of unknown function, was primarily localized to endothelial cells of blood vessels and aqueous outflow structures. Protein staining intensities were markedly reduced in anterior segment tissues of PEX eyes compared to controls and co-localized to LOXL1-positive PEX material deposits on ocular surfaces and in blood vessel walls.
Conclusions: The newly identified loci provide new biological insights into the pathology of PEX syndrome/glaucoma and highlight a role for impaired proteasome function as well as vascular and trabecular endothelial dysfunction in disease pathogenesis.
Commercial Relationships: Matthias Zenkel; **Ursula Schlotzer-Schrehardt**, None; **Daniel Berner**, None; **Friedrich E. Kruse**, None; **André Reis**, None; **Chiea Chuen Khor**, None; **Tin Aung**, None; **Francesca Pasutto**, None

Program Number: 4903 **Poster Board Number:** B0321
Presentation Time: 3:45 PM–5:30 PM
Synergistic protection from high pressure-induced injury by allopregnanolone and 24(S)-hydroxycholesterol in the ex vivo rat retina
Makoto Ishikawa[1], Takeshi Yoshitomi[1], Charles F. Zorumski[2], Yukitoshi Izumi[2]. [1]Ophthalmology, Akita Univ School of Medicine, Akita, Japan; [2]Psychiatry, Washington University School of Medicine, St. Louis, MO.
Purpose: Allopregnanolone (AlloP) and 24(S)-hydroxycholesterol (24SH) are neurosteroids (NS) synthesized in the retina that modulate GABA and glutamate receptors, respectively. We recently reported that these NS are neuroprotective against glaucomatous damage when administered separately at 1 μM. In the present study, we examined whether co-administration of lower concentrations of AlloP (100 nM) and 24SH (100 nM) protect retinal ganglion cells (RGCs) more effectively from intraocular pressure elevation.
Methods: Rat ex vivo eyecups were prepared from 30-day-old male Sprague-Dawley rats. Eyecups were incubated in artificial cerebrospinal fluid (aCSF) at 30°C for 24 hours using a closed pressure system. The pressure in the chamber was increased by introducing 95% O2 and 5% CO2. AlloP (100 nM) and 24SH (100 nM) were separately or simultaneously administered into the aCSF (n=5 for each experiment). Tissue was also processed to determine RGC injury using anti-NeuN antibody (a RGC nuclear marker) and TUNEL staining (n=5 for each experiment). For comparison with controls, we used Dunnett's multiple comparison test.
Results: In whole mounted retinas, the labeling density of anti-NeuN antibody in the RGC (LDN) was 1579.0/mm2 at 75 mmHg (control). Neither 100 nM AlloP alone (LDN=1976.7/mm2, $p>0.05$) nor 100 nM 24SH alone produced significant RGC protection (LDN=1918.1/mm2, $p>0.05$), while co-administration of 100 nM AlloP and 100 nM 24SH significantly promoted survival of NeuN-positive RGCs at 75 mmHg (LDN=2497.0/mm2, $p<0.05$) compared to control. Exposure to elevated pressure induced apoptosis in the GCL and INL (LDA=33.6/200 μm, control). Again, neither 100 nM AlloP alone (LDA=14.2/200 μm, $p>0.05$) nor 100 nM 24SH alone (LDA=13.2/200 μm, $p>0.05$) produced significant protection, while co-administration of 100 nM AlloP and 100 nM 24SH substantially inhibited apoptosis in the retina at 75 mmHg (LDA=0.4/200 μm, $p<0.05$) compared to control.
Conclusions: Co-administration of AlloP and 24SH may serve as effective therapeutic strategies for protecting glaucomatous eyes from pressure-induced injuries.
Commercial Relationships: Makoto Ishikawa, None; **Takeshi Yoshitomi**, None; **Charles F. Zorumski**, Sage Therapeutics (C); **Yukitoshi Izumi**, None

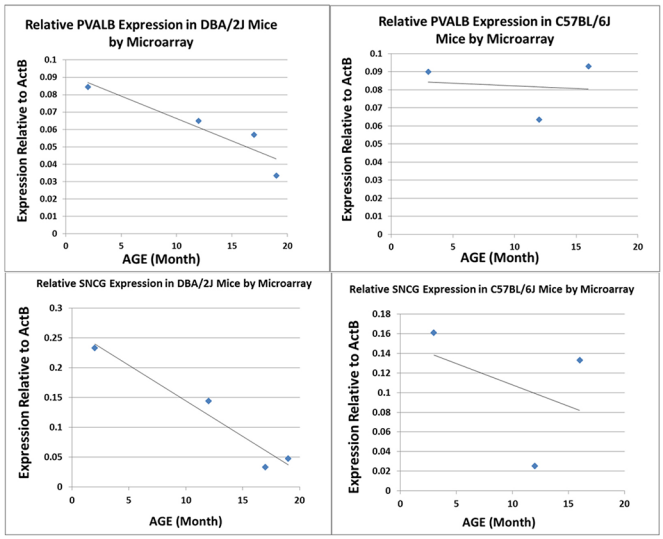
Program Number: 4904 **Poster Board Number:** B0322
Presentation Time: 3:45 PM–5:30 PM
A bioinformatics approach to identify molecular pathways that characterize the trabecular meshwork
Ilona Liesenborghs[1, 2], Carroll Webers[1], Lars Eijssen[3], Martina Kutmon[2, 3], Theo G. Gorgels[1], Wouter Hubens[1], Chris Evelo[3, 2], Henry J. Beckers[1], Johannes Schouten[1]. [1]University Eye Clinic Maastricht, Maastricht University Medical Centre+, Maastricht, Netherlands; [2]Maastricht Centre for Systems Biology (MaCSBio), Maastricht University, Maastricht, Netherlands; [3]Department of Bioinformatics, BiGCaT, Maastricht University, Maastricht, Netherlands.
Purpose: The pathogenesis of primary open angle glaucoma (POAG) has not been clarified and its treatment is still not based on insights in the underlying molecular mechanisms. We performed in silico pathway analysis based on gene expression datasets of healthy human trabecular meshwork (TM) cells. Identification of the active pathways in this tissue contributes to a better insight in the molecular processes and may provide candidate target genes for new treatment options of POAG.
Methods: A search for datasets was conducted in Gene Expression Omnibus and ArrayExpress. We used five microarray datasets (GSE27276, GSE65240, GSE6298, GSE37474, and GSE16643; n=23 individuals), which contained gene expression profiles of healthy trabecular meshwork cells. All data were subjected to a thorough quality control based on ArrayAnalysis.org and R scripts. Divergent samples were removed from the datasets. Pathway analysis and visualisation was conducted with PathVisio using a curated pathway collection from WikiPathways. Ubiquitous pathways (pathways that are common pathways in almost all body tissues) were excluded based on data from TissueAnalyzer. From each dataset, the top 25% highest expressed genes were identified and used for pathway overrepresentation analysis. The top 20 Z-score ranked pathways from the pathway analysis of each dataset were selected as these are likely the most relevant TM active pathways.
Results: Seven ubiquitous pathways were found in the pathway analysis and excluded. One dataset (GE6298) expressed none of these common pathways and did not show any correlation with the results of the other four datasets. Therefore, this dataset was removed from further analysis. Pathways that were expressed in at least three out of four datasets were considered to be active, non-ubiquitous pathways of the healthy TM. Four pathways were significantly expressed in all the datasets and four pathways in three out of four datasets. Several of these pathways have been associated with processes in TM, for example the pathways TNF alpha signalling (WP231), trans-sulfuration (WP2333), and miRNA targets in extracellular matrix and membrane receptor (WP2911).
Conclusions: We identified eight non-ubiquitous pathways that appear to be active in healthy human trabecular meshwork tissue.
Commercial Relationships: Ilona Liesenborghs, None; **Carroll Webers**, None; **Lars Eijssen**, None; **Martina Kutmon**, None; **Theo G. Gorgels**, None; **Wouter Hubens**, None; **Chris Evelo**, None; **Henny J. Beckers**, None; **Johannes Schouten**, None

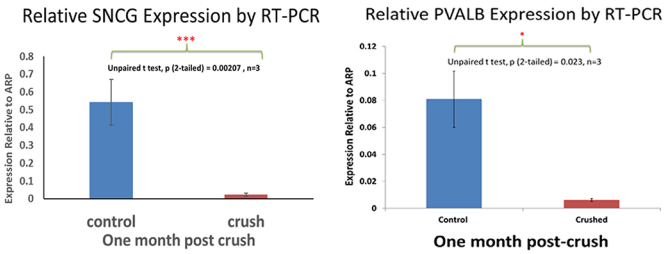
Program Number: 4905 **Poster Board Number:** B0323
Presentation Time: 3:45 PM–5:30 PM
Explore Potential Biomarkers for Retina Ganglion Cell Loss
YUAN LIU, Rossana Cheng He, Mary Tapia, Richard K. Lee. Ophthalmology, Bascom Palmer, Miami, FL.
Purpose: Genetic biomarkers are critical for the early detection of neurodegenerative eye diseases, serving to predict the severity of the disease and its response to treatment. Due to the complexity of neurodegenerative diseases, such as glaucoma, different biomarkers are needed to better study and understand the pathophysiology of these diseases. In this study, two large microarray datasets were used to explore potential biomarkers that would predict the loss of retina ganglion cells.
Methods: Two microarray datasets were used in this project: 1) Data sets derived from 55 different strains of BXD mice and 2) Data sets from DBA/2J and C57BL/6J mice at the age of 3, 12, and 17 months. Retinas from crushed eyes (n=5) and control eyes (n=5) from three-month-odl BL6 mice were used for RT-PCR verification one month after crush. Pooled retinas from DBA/2J and BL6 mice at 3 months (n=8), 12 months (n=8), and 17 months (n=8) were prepared for RT-PCR analysis. Globes of DBA/2J and BL6 mice were fixed in paraformaldehyde and sectioned for immunofluorescence staining

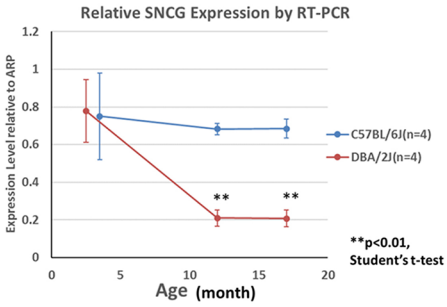
with antibodies against potential RGC markers. Two-tailed Student's t-test was used for statistical analysis.

Results: The expression levels of four candidate genes – sncg, kcnd2, pvalb, and cyfip2 – were found to have a strong correlation with the loss of RGCs based on two microarray datasets. RT-PCR analysis showed that the relative expression levels of sncg and pvalb in crushed eye were significantly lower than those of the control eyes ($p < 0.05$). Similarly, gene microarray analyses, confirmed by RT-PCR, showed a progressive reduction in sncg and pvalb expression in retinas of the DBA/2J glaucomatous mouse compared to their age-matched C57BL/6J control. Furthermore, immunofluorescence microscopy results revealed that in the young and adult mouse retina, sncg is preferentially expressed in RGC somas while pvalb is mainly expressed in RGC dendrites.

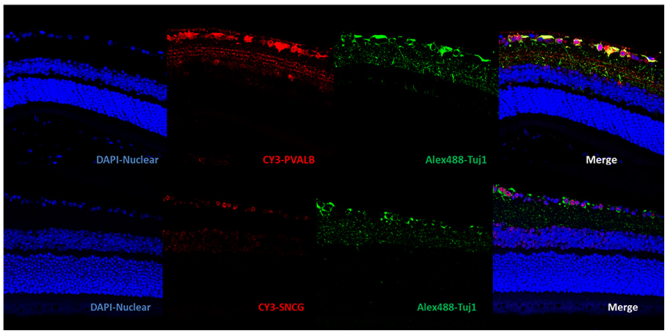
Conclusions: According to the microarray analysis, sncg, kcnd2, pvalb, and cyfip2 are potential RGC markers. RT-PCR results further showed that sncg and pvalb expression levels decreased with the degeneration of RGCs in two different mouse models – the glaucomatous DBA/2J mice and mice that sustained optic nerve crush injury. Additionally, immunofluorescence results demonstrated that sncg and pvalb were specifically expressed in RGCs. In conclusion, the results from this study showed that sncg and pvalb are specific and reliable RGC markers.







Expression of PVALB and SNCG in 3-months C57BL/6J Retinas



Commercial Relationships: YUAN LIU, None; **Rossana Cheng He**, None; **Mary Tapia**, None; **Richard K. Lee**, None

Support: National Glaucoma Research program of the BrightFocus Foundation, unrestricted research grant from Research to Prevent Blindness and NIH center grant EY014801.

Program Number: 4906 **Poster Board Number:** B0324
Presentation Time: 3:45 PM–5:30 PM
Molecular architecture of myocilin and structural defects imparted by non-synonymous mutations in its coiled coil domain
Raquel L. Lieberman, Shannon E. Hill, Elaine Nguyen, Rebecca K. Donegan. School of Chemistry & Biochemistry, Georgia Institute of Technology, Atlanta, GA.
Purpose: Myocilin has been associated with primary open angle and steroid-induced glaucoma for ~20 years. Studies to date have generally focused on disease pathogenesis of mutant myocilins found within the C-terminal olfactomedin domain, which account for ~90% of documented variants and cause amyloid-like protein aggregation. Little is known about the N-terminal coiled coil region in which the remaining ~10% of mutations are located. Coiled coils are known to confer oligomeric states to proteins. We hypothesized that glaucoma-associated mutations in the coiled coil alter the quaternary structure of myocilin, instead of causing aggregation. The purpose of this study was to structurally characterize the myocilin N-terminal domain and assess effects of glaucoma-associated mutations in molecular detail.
Methods: A variety of N-terminal domain constructs were defined and prepared recombinantly, including Cys-->Ser and 7 missense variants documented in genetic studies. Proteins were characterized by a combination of biochemical and biophysical techniques including non-reducing gel electrophoresis, size exclusion chromatography (SEC), circular dichroism melts, and transmission electron microscopy (TEM). Molecular structures were determined by a combination of synchrotron radiation methods including SEC-small-angle X-ray scattering (SEC-SAXS) and X-ray crystallography.
Results: The three Cys residues in the N-terminal domain serve to thermally stabilize the coiled-coil quaternary structure, a defined oligomeric entity whose kinked molecular envelope is defined by SAXS and was confirmed by TEM. The high resolution crystal structure of the C-terminal ~10 kDa of the coiled coil reveals features that are unique when compared to canonical coiled-coils like myosin. Glaucoma-associated variants like L95P exhibit detectable structural defects, but do not result in a protein prone to aggregation.
Conclusions: The extended myocilin architecture is a unique stable entity. In contrast to earlier suggestions, properly folded myocilin is not a disulfide-linked polymer. Misfolding introduced by glaucoma-associated mutations in the coiled-coil region does not result in aggregation but nevertheless impairs trafficking. This work broadens our appreciation for protein misfolding in the pathogenesis of myocilin-associated glaucoma.
Commercial Relationships: Raquel L. Lieberman, None; **Shannon E. Hill**, None; **Elaine Nguyen**, None; **Rebecca K. Donegan**, None
Support: NIH R01EY021205

Program Number: 4907 **Poster Board Number:** B0325
Presentation Time: 3:45 PM–5:30 PM
Structural properties of meningothelial cells in the retrobulbar, mid-orbital and intracanalicular sections of the optic nerve arachnoid mater
Nauke Zeleny[1], Corina Kohler[2], Albert Neutzner[2], Hanspeter E. Killer[1], Peter Meyer[3]. [1]Department of Ophthalmology, Kantonsspital Aarau, Aarau, Switzerland; [2]Department for Biomedicine, Ocular Pharmacology and Physiology, University Hospital Basel, Basel, Switzerland; [3]Department of Ophthalmology, University Hospital Basel, Basel, Switzerland.
Purpose: Alterations of and within the optic nerve microenvironment (ONM) are most likely involved in optic nerve damage in idiopathic intracranial hypertension and normal tension glaucoma. There is increasing evidence that meningothelial cells (MECs) that cover, the arachnoid and the pia mater might be actively involved in homeostatic processes within the subarachnoid space (SAS). However, as MECs have been only poorly characterized with regards to their structural properties we assessed these cells for the presence of gap junction proteins and aquaporin 4 (AQP4) water channels.
Methods: Human meningothelial cells from the arachnoid layer were investigated for the presence of gap junction proteins and aquaporine. For this purpose the MECs were stained for tight junctions (JAM1, occluding, claudin 5), gap junctions (connexin 26 and 43) and desmosomes (desmoplakin) as well as for AQP4 water channels. The retrobulbar, mid-orbital and intracanalicular human optic nerve sections of seven patients (14 optic nerves) with no known optic nerve pathology were processed.
Results: MECs exhibited immunopositivity for markers of tight junctions (JAM1, occluding, claudin 5) and gap junctions (connexin 26 and 43) as well as for waterchannels (AQP4). However no immunopositivity was found for desmoplakin.
Conclusions: Immunohistochemical analyses demonstrate that MECs from the arachnoid layer of the optic nerve express tight junctions and gap junction proteins as well as AQP4 water channels. The expression of tight and gap junction proteins confirm the barrier function previously postulated for MECs. The presence of gap-junctions suggests that MECs work together as a syncytium, thus keeping damage to individual cells in check. Furthermore, AQP4 water channels are likely involved in CSF transport through MECs. These observations provide a novel view on the structural properties of MECs and their involvement for a CSF clearing pathway from the optic nerve microenvironment.
Commercial Relationships: Nauke Zeleny; **Corina Kohler**, None; **Albert Neutzner**, None; **Hanspeter E. Killer**, None; **Peter Meyer**, None

Program Number: 4908 **Poster Board Number:** B0326
Presentation Time: 3:45 PM–5:30 PM
Stabilization Of Prdx6 Activity By Targeted Mutation Of Sumoylation Sites Protects Trabecular Meshwork Cells From Oxidative Damage
Dhirendra P. Singh[1], Bhavana Chhunchha[1], Prerna Singh[1], W Daniel Stamer[2], Eri Kubo[3]. [1]Ophthalmology and Visual Sciences, Univ of Neb Med Center, Omaha, NE; [2]Ophthalmology, Duke Eye Center, Duke University, Durham, NC; [3]Ophthalmology, Kanazawa Medical University, Kanazawa, Japan.
Purpose: Abnormalities in trabecular meshwork (TM) caused by oxidative stress may lead to higher intraocular pressure and progression of glaucoma. We previously showed the involvement of aberrant Sumoylation of the antioxidant protein, peroxiredoxin (Prdx)6 in the pathobiology of TM cells. Here we test whether targeted mutation of Sumoylation sites in Prdx6 protects TM cells from oxidative damage.
Methods: TM cells isolated from one glaucomatous and seven normal donor eyes were exposed to H_2O_2 (0-200μM) for variable times. Subjects were in three age groups: young (3, 11 m), middle (39, 51, 54 y) and old (79, 88 y), plus glaucoma (56y). We assessed the effect of Prdx6 Sumoylation on ECM proteins, senescence markers p16/p21, by Sumo/Prdx6-ELISA, Western analysis, and qPCR, and examined correlation of these parameters with pathobiology of TM cell, if any. Prdx6 cDNA and mutant Prdx6 cDNA [at Sumo1 sites K (lysine) 122/142R (arginine) by site-directed mutagenesis] were cloned into the pTAT-vector. Purified TAT-Prdx6 protein was transduced into TM cells to assess its efficacy in abating pathobiology of cells overexpressing pSumo1. SA-βgal, MTS and H2DCF-DA dye assays measured cell senescence, viability and reactive oxygen species (ROS). The GSH-peroxidase

and aiPLA2 activities were done by commercial kits. A two tailed Student's t-test was used for statistical analysis.
Results: TM cells from the middle and old groups plus the glaucomatous subject showed reduced Prdx6 levels and increased Sumoylation of Prdx6 compared to younger subjects ($p<0.001$). The reduced Prdx6 activity was linked to increased ROS, SA-βgal activity, Sumo1 and ECM expression ($p<0.05$). The process was significantly increased (~3fold) in aged or redox-active cells due to loss of Prdx6 activity, mediated by aberrant Sumoylation ($p<0.001$). Cells transduced with Prdx6K122/142R were protected from oxidative damage having augmented GSH Peroxidase and aiPLA$_2$ activities. Moreover, TAT-Prdx6 K122/142R transduced TM cells, reversed ROS overshooting and abated TM cell injuries and senescence ($p<0.001$).
Conclusions: Findings reveal a mechanism regulated by Sumoylation of Prdx6 that rescued cells from oxidative stress-induced damage, suggesting a therapeutic potential for Sumoylation-deficient Prdx6 and offering a novel strategy for the treatment of glaucoma.
Commercial Relationships: Dhirendra P. Singh, None; **Bhavana Chhunchha**, None; **Prerna Singh**, None; **W Daniel Stamer**, None; **Eri Kubo**, None
Support: BrightFocus Foundation, G2014067 and NEI, EY024589

Program Number: 4909 **Poster Board Number:** B0327
Presentation Time: 3:45 PM–5:30 PM
Alternative splicing and nonsense-mediated mRNA decay contribute to regulation of LOXL1 expression in response to cellular stress in pseudoexfoliation
Daniel Berner[1], Matthias Zenkel[1], Francesca Pasutto[2], Johannes Schödel[3], André Reis[2], Friedrich E. Kruse[1], Ursula Schlotzer-Schrehardt[1]. [1]Department of Ophthalmology, University of Erlangen-Nürnberg, Erlangen, Germany; [2]Institute of Human Genetics, University of Erlangen-Nürnberg, Erlangen, Germany; [3]Department of Nephrology and Hypertension, University of Erlangen-Nürnberg, Erlangen, Germany.
Purpose: Alternative mRNA splicing coupled to nonsense-mediated decay (NMD) is a common mRNA surveillance pathway also known to dynamically modulate gene expression in response to cellular stress. Here, we investigated the involvement of this pathway in regulation of lysyl oxidase-like 1 (LOXL1) expression in response to pseudoexfoliation (PEX)-associated stress factors.
Methods: Transcript levels of *LOXL1* isoforms were determined by qPCR using specific primers in ocular tissues obtained from donor eyes without and with PEX syndrome. Human Tenon's capsule fibroblasts (hTCF), trabecular meshwork cells (hTMC), lens epithelial cells (hLEPC), and optic nerve head astrocytes (hONHA) were exposed to puromycin, emetine, caffeine, TGF-ß1, homocysteine, interleukin-6, retinoic acid, UVB-radiation, oxidative and mechanical stress for up to 48 hours. siRNA mediated silencing of UPF1 (regulator of nonsense transcripts 1) was performed in hTCF.
Results: ENSEMBL database analysis indicated the presence of an alternative *LOXL1* transcript (*LOXL1*-002) characterized by inclusion of an additional exon X between exon 1 and 2 introducing a premature termination codon in exon 2. *LOXL1-002* transcripts were detected in all ocular tissues showing highest expression levels in trabecular meshwork, lens and lamina cribrosa, with significant differences between PEX and control tissues. Treatment of hTCF, hTMC, hLEPC and hONHA with NMD inhibitors puromycin, emetine and caffeine significantly increased *LOXL1-002* mRNA levels, but reduced levels of wild-type *LOXL1* mRNA. Significantly enhanced *LOXL1-002* expression upon knockdown of UPF1, a key regulator of NMD pathway, further confirmed that *LOXL1-002* is a direct target of NMD. Exposure of cells to various PEX-associated (stress) factors, including TGF-ß1, retinoic acid, UV light, oxidative and mechanical stress, resulted in upregulated *LOXL1-002* expression in a cell type-specific manner and altered steady state levels of wild-type *LOXL1* expression.
Conclusions: These findings indicate that alternative splicing coupled to NMD is dynamically modulated by cellular stress and contributes to post-transcriptional regulation of *LOXL1* expression in the pathogenesis of PEX syndrome.
Commercial Relationships: Daniel Berner, None; **Matthias Zenkel**, None; **Francesca Pasutto**, None; **Johannes Schödel**, None; **André Reis**, None; **Friedrich E. Kruse**, None; **Ursula Schlotzer-Schrehardt**, None
Support: Interdisciplinary Center for Clinical Research (IZKF), Erlangen, Germany

Program Number: 4910 **Poster Board Number:** B0328
Presentation Time: 3:45 PM–5:30 PM
Proteomics Analysis of LOXL1-containing Exosomes Secreted by Len Epithelial Cells
Jingwen Cai, Mariam L. Khaled, Yutao Liu. Cellular Biology and Anatomy, Augusta University, Augusta, GA.
Purpose: Exfoliation syndrome (XFS) is the most common cause of secondary glaucoma. XFS is characteristic with the accumulation of exfoliation material in the anterior chamber including lens. Several environmental factors including TGF-b, IL-6, hyperhomocysteine, and oxidative stress, may contribute to the pathogenesis of XFS. We aimed to study how these environmental factors affect the proteomic content of exosomes secreted by lens epithelial cells.
Methods: Lens epithelial cell line B3 reached 80% confluence before changing to serum-free condition media at different conditions, non-treated control, TGF-b (10ng/ml), IL-6 (50ng/ml), hyperhomocysteine (500mM), and H_2O_2 (250mM). Exosomes were isolated using the Total Exosome Isolation Reagent from Invitrogen. The isolated exosomes were characterized using nanoparticle tracking analysis (NTA) with ZetaView for their size and particle concentration. Exosomes was freeze-dry and lysed for proteomics analysis using mass spectrometry after trypsin digestion. Lists of measured peptide masses were used to identify the protein components in the isolated exosomes. Extracellular proteins including LOXL1 and its interaction partners were examined. Levels of LOXL1 protein in cellular lysate from B3 cells under the four treatment conditions were examined using ELISA assay.
Results: NTA data indicated the isolated exosomes with a typical size distribution (50-150 nm). Proteomics analysis indicated the presence of several exosome protein markers, including CD44, CD81, FLOT1, and HSPA8. While LOXL1 protein was not actively secreted under normal condition, IL-6 treatment increased secretion of LOXL1 into exosomes. Other extracellular proteins[l1], such as EFEMP1 and DCN, were increased under different treatments. IL-6 and TGF-β treatments promoted the secretion of FBLN5 and RELN, while hyperhomocysteine and oxidative stress induced the secretion of FBLN1, LTBP1, and BMP1. Secretion of fibronectin was dramatically increased with IL-6 treatment. [l2] The results from the ELISA assay for LOXL1 indicated that production of LOXL1 significantly increased under treatment of IL-6 and H_2O_2.
Conclusions: We have characterized the protein components in secreted exosomes from lens epithelial cells under the influence of XFS-related environmental factors. This study will advance our understanding of the molecular mechanism of XFS.
Commercial Relationships: Jingwen Cai; **Mariam L. Khaled**, None; **Yutao Liu**, None

Support: The Glaucoma Foundation, Glaucoma Research Foundation, BrightFocus Foundation

Program Number: 4911 **Poster Board Number:** B0329
Presentation Time: 3:45 PM–5:30 PM
Evaluation of the potential of iPSCs as a tool to study Pseudoexfoliation Syndrome
Coralia C. Luna, Megan Parker, Pratap Challa, Pedro Gonzalez. Ophthalmology, Duke University, Durham, NC.
Purpose: A major limitation to investigate the pathophysiology of pseudoexfoliation (PEX) syndrome and develop effective treatments is the lack of experimental models that recapitulate the main alterations in production and maturation of extracellular material observed in PEX patients. The objective of this research was to test the potential of induced pluripotent stem cells (iPSCs) from PEX donors to generate an *in vitro* model for PEX syndrome.
Methods: Primary cultures of dermal fibroblasts were generated from skin biopsies from 2 confirmed PEX donors and reprogramed into iPSCs by transfection with plasmids: Oct4+P53 inhibitor, Sox2, Klf4, L-Myc, Lin 28 and Ebna. Reprograming of selected clones was confirmed by immunofluorescence. The iPSCs were differentiated into vascular smooth muscle cells or vascular endothelial cells by incubation in specific differentiation media and differentiation levels were evaluated by western blot of SMA and SM22 for smooth muscle, and VECAD and CD31 for vascular endothelial cells. iPSCs procured from the NINDS Repository from 4 non-PEX donors were used as controls and differentiated under the same conditions. Differentiated cultures were examined by transmission electron microscopy at 7 and 14 days after reaching confluency to evaluate production of microfibrils and maturation of elastic fibers.
Results: iPSCs from both control and PEX donors could be differentiated into smooth muscle and vascular endothelial cells with levels of expression of differentiation markers that varied among the cell lines particularly for vascular endothelial cells. Both cell types showed extensive production of basement membrane components including microfibrils and elastic fibers. In absence of any additional stressors or regulatory factors, cells form PEX donors showed more microfibrils than controls and, although formed thicker electron dense bundles, did not mature into normal elastic fibers suggesting differences in elastogenesis between cells derived from PEX donors and controls.
Conclusions: The observed extensive production of basal membrane components and differences in elastogenesis between PEX and control cells suggest that differentiation of iPSCs from PEX donors into cell types capable of forming basement membranes, such as smooth muscle and vascular endothelial cells, can be a useful approach to develop an *in vitro* experimental model to investigate the molecular pathogenesis of PEX syndrome.
Commercial Relationships: Coralia C. Luna, None; **Megan Parker**, None; **Pratap Challa**, None; **Pedro Gonzalez**, None
Support: NH Grant EY23287

Program Number: 4912 **Poster Board Number:** B0330
Presentation Time: 3:45 PM–5:30 PM
Expression of Acid-Sensing Ion Channels in Optic Nerve Astrocytes
Tara Tovar-Vidales[1], Yang Liu[1], Eric B. Gonzales[2], Abbot F. Clark[1]. [1]NTERI, University of North Texas Hlth Sci Ctr, Fort Worth, TX; [2]Center for Neuroscience Discovery, Institute for Healthy Aging, University of North Texas Hlth Sci Ctr, Fort Worth, TX.
Purpose: Ion channels have been associated in the pathology of fibrotic diseases including glaucoma. We examined the expression of acid-sensing ion channels (ASIC) 1a, 1b, 2a, and 2b in mouse optic nerve astrocytes (ONA). The purpose of this study was to determine: (a) whether ASIC channels are expressed in ONA, (b) whether expressions of these ASIC channels are regulated by TGF-β2, (c) whether the functional expression of ASIC1a are present in ONA.
Methods: Primary mouse ONA were obtained from C57BL/6 mice. ONA were treated with or without TGF-β2 (5ng/ml) for 24 hours. Immunocytochemistry was used to determine expression and localization of ASIC channels in ONA. Western blot analysis of cell lysate was used to determine if TGF-β2 increased expression of ASIC channels by primary mouse ONA. ONA were subjected to whole-cell patch-clamp electrophysiology to detect amiloride-sensitive ASIC currents.
Results: ASIC1a and ASIC2a proteins are present in mouse ONA. ASIC1a was localized to the cytoplasm and perinucleus by immunocytochemistry. TGF-β2 increased ASIC1a protein expression by Western immunoblot. ASIC 2a was localized to the cytoplasm by immunocytochemistry. ASIC1b and ASIC2b were not detected in ONA by immunocytochemistry or Western blot. Whole-cell patch-clamp electrophysiology confirmed the presence of amiloride-sensitive ASIC currents in ONA.
Conclusions: These data indicate that ASIC channels are expressed by ONA and may be regulated by TGF-β2. Therefore, TGF-β2 may be involved in altered ASIC function and subsequently in ONA fibrosis.
Commercial Relationships: Tara Tovar-Vidales, None; **Yang Liu**, None; **Eric B. Gonzales**, None; **Abbot F. Clark**, NiCox Research Institute (F), Western Commerce (C), Reata Pharmaceuticals (F)

Program Number: 4913 **Poster Board Number:** B0331
Presentation Time: 3:45 PM–5:30 PM
TXNRD2 and CDKN2B-AS1 gene polymorphisms in patients with Primary Open-Angle Glaucoma
Artur L. Tenório[1, 2], Rodrigo F. do Carmo[4], Rodrigo P. Lira[2], Roberto P. Galvao Filho[1], Pedro T. Falcão Neto[1], Rinalva T. Vaz[6], Walter L. Barbosa Junior[3], Lucas D. e Silva[3], Raul E. de Lima[3], André L. Tenório[5], Claudia M. Figueiroa[5], Isabela C. de Macedo[5], Julia N. Monteiro[5], Gustavo H. Melo[5], Vitor C. Lima[5], Luydson R. Vasconcelos[3]. [1]Recife Eye Institute, Recife, Brazil; [2]Ophtalmology, Federal University of Pernambuco, Recife, Brazil; [3]Reserch Center Aggeu Magalhães, Recife, Brazil; [4]Federal University of São Francisco Valley, Petrolina, Brazil; [5]University of Pernambuco, Recife, Brazil; [6]Santa Luzia Eye Hospital, Recife, Brazil.
Purpose:
Recent studies have demonstrated association between single nucleotide polymorphisms (SNPs) in TXNRD2 and CDKN2B-AS1 genes and primary open angle glaucoma (POAG) in some populations. This is the first study to determine the frequency of these SNPs in a Brazilian's population with POAG.
Methods: We performed a retrospective, case-control study to confirm the association between the rs35934224 (TXNRD2) and rs7866783 (CDKN2B-AS1) and POAG in a population from the northeast region of Brazil. A total of 156 individuals were enrolled, being 87 cases with POAG (43 males and 44 females) and 73 controls (27males and 46 females) from the Recife Eye Institute. Mean age was 67,5 years for cases and 67,7 years for controls. Cases were primarily defined as individuals with at least one reliable visual field showing vision loss consistent with glaucoma or documented progression of optic nerve degeneration by OCT-Spectralis, with Cup-to-Disc ratio ≥0.7. Controls had CDR <0.6, IOP <17 mmHg and family history negative for glaucoma. Genomic DNA was isolated using commercially available kits and detection of the SNPs was done by Real Time PCR using TaqMan probes. Differences in the

frequency distribution between the groups were analyzed using the chi-squared test or Fisher's exact test when appropriate.
Results: Individuals with POAG had a higher frequency of the CC genotype (rs35934224) in the TXNRD2 gene than controls (71.0% vs. 61.1%, p=0.18). The C allele presented similar distribution (84.3% vs. 80.5%, p=0.38). Regarding the rs7866783 in the CDKN2B-AS1 gene, the GG genotype was more prevalent in individuals with POAG (67.8% vs. 63.0%, p=0.52). The G allele was equally distributed (82.1% vs. 78.7%, p=0.44).
Conclusions: No association was observed between the CC and GG risk genotypes in the TXNRD2 and CDKN2B-AS1 genes, respectively, with POAG in Brazilian patients. Further studies are needed to confirm these results in a larger cohort.
Commercial Relationships: Artur L. Tenório; **Rodrigo F. do Carmo**, None; **Rodrigo P. Lira**, None; **Roberto P. Galvao Filho**, None; **Pedro T. Falcão Neto**, None; **Rinalva T. Vaz**, None; **Walter L. Barbosa Junior**, None; **Lucas D. e Silva**, None; **Raul E. de Lima**, None; **André L. Tenório**, None; **Claudia M. Figueiroa**, None; **Isabela C. de Macedo**, None; **Julia N. Monteiro**, None; **Gustavo H. Melo**, None; **Vitor C. Lima**, None; **Luydson R. Vasconcelos**, None

Program Number: 4914 **Poster Board Number:** B0332
Presentation Time: 3:45 PM–5:30 PM
Differential purification of retinal ganglion cells from human induced pluripotent stem cell derived 3D retinal organoids
Wataru Kobayashi[1, 2], Akishi Onishi[1], Sunao Sugita[1], Kyoko Iseki[1], Yuji Takihara[3], Masaru Inatani[3], Toru Nakazawa[2], Masayo Takahashi[1]. [1]Retinal regeneration, RIKEN Center for developmental biology, Kobe, Japan; [2]Department of Ophthalmology, Tohoku University, Sendai, Japan; [3]Department of Ophthalmology, Faculty of Medical Science, University of Fukui, Fukui, Japan.
Purpose: Retinal ganglion (RG) cells are traditionally difficult to study from both tissue explant and human induced pluripotent stem (iPS) cell derived 3D retinal organoids. Therefore, a method to efficiently purify RG cells from large 3D retinal organoid differentiation may help to overcome current limitations. In this study, we compared cell populations and sorting quality between the two-step immunopanning method and the MACS (Magnetic-Activated Cell Sorting) system.
Methods: Human 3D retinal organoids differentiated from 201B7 iPS cells were maintained for 50 to 75 days. In the two-step immunopanning method, dissociated RG cells were purified by anti-macrophage and anti-Thy 1.1 antibodies. In the MACS system, the dissociated RG cells were purified by magnetic microbeads conjugated to anti-Thy1 antibodies. The purified iPS cell derived RG cells (iPS-RG) cells were then plated on dishes coated with poly-D-lysine and laminin, and were maintained in serum-free medium with neurotrophic factors. The growth and morphology of the neurites were visualized by immunocytochemistry with BRN3B and SMI-312 antibodies.
Results: The iPS-RG cells purified by both immunopanning and MACS methods attached on to dishes in the first 24 hours and extended neurites. Our iPS-RG cells from either method could be cultured for about three weeks. One to three days after plating, the RG cells purified by MACS grow faster than those purified by immunopanning. Conversely, there were more cells with glia-like morphology retained in MACS-purified cell cultures. Interestingly, neurites of iPS-RG cells were similar between assays, yet the length of the neurites from MACS purified iPS-RG cells was longer.
Conclusions: We validated the neurite growth of human iPS-RG cells purified with two distinct methods and cultured over an extended period. The purity of iPS-RG cells by MACS system was lower than the two-step immunopanning method, but the glia-like cells may positively influence on neuritogenesis of purified iPS-RG cells.
Commercial Relationships: Wataru Kobayashi, None; **Akishi Onishi**, None; **Sunao Sugita**, None; **Kyoko Iseki**, None; **Yuji Takihara**, None; **Masaru Inatani**, None; **Toru Nakazawa**, None; **Masayo Takahashi**, None

Program Number: 4915 **Poster Board Number:** B0333
Presentation Time: 3:45 PM–5:30 PM
MiR-214-3p/GUCA1A axis contributes to the development of glaucoma in a mouse model?
Lu Lu[1], Qingqing Gu[2], Byron Jones[1], Robert Williams[1], Junming Yue[2]. [1]Department of Genetics, Genomics, and Informatics, University of Tennessee Health Science Center, Memphis, TN; [2]Department of Pathology, University of Tennessee Health Science Center, Memphis, TN.
Purpose: miRNA is a class of endogenous, small RNAs that inhibit gene expression at the post-transcriptional level. Accumulating evidence indicates that miRNAs are involved in ocular diseases including glaucoma, neovascularization, retinitis pigmentosa, retinoblastoma, etc. Glaucoma, characterized by the loss of retinal ganglion cells, is the second leading cause of blindness in the United States. This study investigates the role of miRNAs in the development of glaucoma.
Methods: The fresh retinas were harvested from DBA/2J (D2) mice--an animal model of glaucoma, C57BL/6J (B6) mice--a glaucoma-resistant mouse, and DBA/2J-Gpnmb wild-type (D2WT) mice that are glaucoma-resistant at different aging stages. The expression data of miRNAs and mRNA were collected using Affymetrix Mouse GeneChip® miRNA Arrays 4.0 and Affymetrix Mouse Gene ST V1.0 respectively. Two-tailed Student's t-test was used for statistical analysis.
Results: We observed more than 100 miRNAs that showed differential expression between D2 and B6, and between D2 and D2WT mice during different developmental stages. Several miRNAs showed significant increased expression including miR-214, miR-29, miR-9, miR-24 in D2 mice at an onset of glaucoma (7-9 months) compared to early stages (2-4 months); the same miRNAs in B6 and D2WT mice did not show significant changes in expression at the same stage. Interestingly, we found that expression of Guca1a, a potential target of miR-214-3p, was reduced in D2 mice while it was not significantly altered in B6 and D2WT mice. It is known that Guca1a mutation is the major cause of photoreceptor degeneration.
Conclusions: Our results indicate that miR-214-3p may be a potential biomarker of glaucoma and other retinal degeneration diseases. In addition, this study uncovered a novel molecular mechanism underlying glaucoma, and provides a new avenue to study the potential therapy for glaucoma.
Commercial Relationships: Lu Lu; **Qingqing Gu**, None; **Byron Jones**, None; **Robert Williams**, None; **Junming Yue**, None
Support: EY021200

Program Number: 4916 **Poster Board Number:** B0334
Presentation Time: 3:45 PM–5:30 PM
Genome-wide transcriptome profiling of human trabecular meshwork cells treated with TGFβ-2
Colin E. Willoughby[1, 2], Karen Lester[1], Kasia Whysall[3], Anshoo Choudhary[2, 1], Kevin J. Hamill[1], Carl Sheridan[1]. [1]Dept of Eye and Vision Science, University of Liverpool, Liverpool, United Kingdom; [2]St. Paul's Eye Unit, Royal Liverpool University Hospital, Liverpool, United Kingdom; [3]Institute of Ageing and Chronic Disease, University of Liverpool, Liverpool, United Kingdom.

Purpose: Transforming growth factor beta 2 (TGFβ-2) is elevated in the aqueous humour of primary open angle glaucoma (POAG) patients and results in structural and molecular changes in the human trabecular meshwork (TM). Identifying specific effectors of the TGF-ß2 response in the TM will facilitate the identification of new molecular targets for therapeutic manipulation. RNA-Seq was performed on cultured normal human TM cells treated with TGFβ-2 in order to profile genome-wide transcriptional alterations and to identify the genes and gene-gene networks dysregulated by a known glaucoma stimulus.
Methods: Primary normal human TM cells (n=5) were grown to confluence and treated with 5ng/mL TGFβ-2 in serum free low glucose DMEM for 24 hours. Total RNA was extracted using Qiagen AllPrep and RNA quality checked on the Agilent Tapestation. RNA-Seq was performed with 50 bp paired end reads (60 million reads/sample) using the Illumina NextSeq500 by Exiqon. Bioinformatics analysis used a combination of analysis components (Tuxedo, Bowtie2, Tophat and Cufflinks). Gene ontology enrichment analysis was performed with a standard Fisher's test and the 'Elim' method. Realtime-qPCR was performed on Lightcycler480 system (Roche) to validate 10 of the most significant differentially expressed genes.
Results: The read quality was high: 99.9% of reads had a Q score >30. 46.3 million reads were obtained from each sample and the average genome mapping was 82%. Differentially expressed genes (DEGs) were ranked by logFC and p values. A number of key regulators of the TGFβ and Wnt signalling pathways were in the significantly altered DEGs and were validated by qPCR. Gene ontology enrichment analysis of the differentially expressed genes identified extracellular matrix (ECM) organization (GO:0030198) as a key pathway altered by TGFβ-2 in human TM cells.
Conclusions: RNA-Seq is a powerful tool to investigate genome-wide alterations in gene expression in TM treated with a known glaucoma stimulus (TGFβ-2) as a hypothesis-independent approach and implicates alterations in ECM organization in POAG pathogenesis. RNA-Seq can identify therapeutic targets for future molecular therapies to treat the TM in POAG and/or prevent TGF-β2 induced scarring following glaucoma filtration surgery.
Commercial Relationships: Colin E. Willoughby, None; **Karen Lester**, None; **Kasia Whysall**, None; **Anshoo Choudhary**, None; **Kevin J. Hamill**, None; **Carl Sheridan**, None
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Program Number: 4917 **Poster Board Number:** B0335
Presentation Time: 3:45 PM–5:30 PM
Angiotensin II type 1 receptor blockers lower IOP in mice and reduce TGFβ signaling in retinal ganglion cells
Ralph J. Hazlewood, John Kuchtey, Rachel W. Kuchtey. Ophthalmology and Visual Sciences, Vanderbilt University Medical Center, Nashville, TN.
Purpose: Angiotensin II type 1 receptor blockers (ARBs) are commonly used to treat systemic hypertension. In addition to interfering with the renin-angiotensin system (RAS), ARBs attenuate signaling via TGFβ. ARBs also exhibit neuroprotective effects in glaucoma models, but their ability to lower IOP is debated. Currently, there are eight ARBs in clinical use in the US, each with different chemical structures, active metabolites, and different modes of binding to angiotensin II type 1 receptor (AT1R). The purpose of this study is to investigate the IOP lowering capability of ARBs as potential treatments for glaucoma.
Methods: The ARBs losartan, irbesartan, telmisartan, or vehicle controls were administered orally to 3 month-old C57BL/6 mice for 7 days. Before and after treatment, IOP was measured by TonoLab rebound tonometry and blood pressure (BP) was measured by tail-cuff method. Following measurements, mice were sacrificed and eyes were enucleated and processed for immunohistochemistry (IHC) for components of the RAS pathway.
Results: BP was significantly reduced in all ARB treatment groups. Expression of AT1R, renin, and phosphorylated SMAD2 (a marker of TGFβ signaling) was altered in the retina following ARB treatment. IOP was not affected by losartan administration after 3 days and 7 days. However, IOP was significantly reduced after 3 days and 7 days of treatment with irbesartan ($p < 0.001$, $n = 14$ and $p < 10^{-4}$, $n = 8$, respectively) as well as after treatment with telmisartan (3 days $p < 10^{-4}$, $n = 12$ and 7 days $p < 0.05$, $n = 8$).
Conclusions: Oral administration of ARBs results in physiologically relevant drug concentrations, as evidenced by lower systemic BP and changes in RAS component expression in the eye after treatment with all ARBs tested. ARB administration lowers TGFβ signaling in the eye, as has been shown for other tissues. IOP-lowering capability varies by specific ARB used, with losartan having no effect, in agreement with previous results. On the other hand, telmisartan and irbesartan administration results in significant lowering of IOP. Considering that TGFβ is elevated in glaucoma subjects, combined with inhibitory effects on TGFβ signaling, ARBs with IOP-reducing capabilities may be novel treatments for glaucoma.
Commercial Relationships: Ralph J. Hazlewood, None; **John Kuchtey**, None; **Rachel W. Kuchtey**, None
Support: NIH Grant EY020894 & EY008126, Marfan Foundation

Program Number: 4918 **Poster Board Number:** B0336
Presentation Time: 3:45 PM–5:30 PM
Expression of fibrotic and inflammation markers in the human glaucomatous optic nerve head
Oliver W. Gramlich[1, 2], Dina F. Ahram[1], Markus H. Kuehn[1, 2]. [1]Dept. of Ophthalmology & Visual Sciences, The University of Iowa, Iowa City, IA; [2]Center for the Prevention and Treatment of Visual Loss, Iowa City VA Health Care, Iowa City, IA.
Purpose: Structural and compositional changes of the optic nerve head (ONH) are a hallmark of glaucoma. This study aims to compare gene expression profiles in ONHs of human donors with primary open angle glaucoma (POAG) and unaffected controls to identify pathways functionally related to tissue remodeling and inflammation.
Methods: ONHs were collected from patients with clinically confirmed POAG (n=6) and age-matched control donors (n=6). RNA was extracted from ONHs and assayed using Affymetrix Human Exon 1.0 ST arrays. Differentially expressed genes in glaucoma versus control were identified by ANOVA. Quantitative RT-PCR and immunohistochemistry were performed to validate selected differentially expressed genes.
Results: Microarray analysis revealed 144 genes with reduced and 225 genes with elevated expression in ONHs of POAG donors when compared to controls. Pathway analysis indicates that many of these genes are functionally related to tissue remodeling (43 genes; p=0.015), proliferation (15 genes; p=0.045) and cellular inflammation (5 genes; p=0.028). PTGS2, CMKlR1, NMI, ITGBL1 and FBLN5 are representative markers of these groups and their heightened expression in POAG was verified by RT-PCR (1.2 to 5.4 fold increase). Immunohistochemistry indicated increased expression of FBLN5 as well as signs of cellular infiltrates in the nerve fiber layer and the lamina cribosa of glaucomatous ONHs.
Conclusions: As expected, the glaucomatous changes to the human ONH are associated with altered expression levels of a large number of genes. Interestingly, our data suggest increased expression of both inflammation-related genes and those associated with a fibrotic

response. These processes have frequently been observed in concert with one another during CNS injury and are capable of reciprocal stimulation. Fibrosis can engender changes in the biophysical properties of the glaucomatous ONH and will present a barrier to axonal regeneration. The degree to which these events contribute to glaucomatous damage remains to be examined.
Commercial Relationships: Oliver W. Gramlich; **Dina F. Ahram**, None; **Markus H. Kuehn**, None

Program Number: 4919 **Poster Board Number:** B0337
Presentation Time: 3:45 PM–5:30 PM
Characterization of the RNA-binding protein Rbfox1 in the Mouse Retina
Lei Gu, Joseph Caprioli, Natik Piri. Ophthalmology, University of California Los Angeles, Los Angeles, CA.
Purpose: The Rbfox1 (RNA-binding protein, fox-1 homolog) splicing and transcription factor has been identified earlier with gene expression profiling of retinal ganglion cells (RGCs). The present study evaluates the expression pattern of Rbfox1 in developing and adult mouse retina as well as its function in Rbfox1 knockout animals.
Methods: E12, E15, P0, P5, P21 and adult wild type mouse retinas were used for the cellular localization of Rbfox1 protein. Retinal whole mount immunostaining was used for quantitative analysis of Rbfox1-positive cells. Adult Rbfox1loxP/loxP/Ubiquitin-$Cre^{+/-}$ transgenic mice were used for tamoxifen-induced conditional deletion of the Rbfox1. The effect of Rbfox1 ablation on retinal morphology and cell numbers was analyzed eight weeks after tamoxifen administration. The functional integrity of the retino-geniculo-cortical pathway was evaluated with the visual cliff test.
Results: Rbfox1 immunoreactivity was observed in the ganglion cell layer (GCL) of the developing retina at E12, and became more prominent at E15. Rbfox1 immunolabelling extended from the GCL to the inner nuclear layer (INL) at P0 and starting at P5, Rbfox1-expressing cells were clearly colocalized with Rbpms-positive RGCs in the GCL and calbindin-positive amacrine cells (ACs) in the GCL and INL. Whole mount immunostaining of the adult mouse retinas showed that very nearly 100% of RGCs and ACs express Rbfox1. Conditional deletion of the Rbfox1 was used to evaluate the function of this gene in the retina. $Rbfox1^{loxP/loxP}$/Ubiquitin-$Cre^{+/-}$ mice were viable with normal size and weight but had reduced fertility compared to $Rbfox1^{+/-}$ or wild-type littermates. As expected, Rbfox1 expression in RGCs and ACs of $Rbfox1^{-/-}$ animals was dramatically reduced. No evidence of morphological changes in the retinas of Rbfox1 knockout mice was observed and the average RGC and AC densities in these animals were comparable to that of controls. However, the sensitivity of visual depth perception of $Rbfox1^{-/-}$ mice was reduced by approximately 75%.
Conclusions: Rbfox1 expression in RGCs and ACs starts at early stages of their differentiation and remains strong in these cells in fully developed retinas. Although no morphological changes in the retinas of transgenic animals were detected eight weeks after deletion of Rbfox1, the deficiency of depth perception in these animals suggests a functional importance of this gene in vision processing.
Commercial Relationships: Lei Gu, None; **Joseph Caprioli**, None; **Natik Piri**, None
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