476 Ocular Transcriptomics and proteomics
Wednesday, May 10, 2017 3:45 PM–5:30 PM
Room 314 Paper Session
Program #/Board # Range: 4772–4778
Organizing Section: Biochemistry/Molecular Biology

Program Number: 4772
Presentation Time: 3:45 PM–4:00 PM
Transcriptome analysis of AMD retina reveals convergent molecular pathology
Rinki Ratnapriya[1], Margaret R. Starostik[1], Rebecca Kapphahn[2], Ashley Walton[1], Alexandra Pietraszkiewicz[1], Matthew Brooks[1], Sandra R. Montezuma[2], Lars Fritsche[3], Goncalo Abecasis[3], Deborah A. Ferrington[2], Anand Swaroop[1]. [1]Neurobiol-Neurodegen & Repair Lab, NEI, Bethesda, MD; [2]Department of Ophthalmology and Visual Neurosciences, University of Minnesota, Minneapolis, MN; [3]Center for Statistical Genetics, Department of Biostatistics, University of Micgigan, Ann Arbor, MI.
Purpose: AMD has seen spectacular success in deciphering the genetic underpinnings with 52 common and rare variants at 34 loci identified to date. Yet, the mechanism through which associated variants confer liability has not been extensively explored. Post-GWAS, variation in gene expression regulation has been established as a major basis of complex disease risk. However, a comprehensive analysis of gene expression and regulation in healthy and diseased retina has been lacking in the field. Here we describe transcriptome analysis (RNAseq) and whole genome genotyping of human retinae of over 500 donor retinas at different stages of AMD to address some of these challenges.
Methods: Donor eyes were evaluated for absence/presence and severity of AMD using the Minnesota Grading Systems. Retina samples from 128 controls, 197 early AMD, 126 intermediate AMD and 66 advanced AMD of were analyzed. RNA and DNA was isolated using TRIzol®, libraries were made with the TruSeq® Stranded mRNA Library Preparation Kit and sequencing was performed on the Illumina Hiseq 2500. Reads were aligned to the Ensembl 38.5 using STAR, and RSEM was used to estimate gene- and transcript level quantification. Differential analysis was performed using limma; DAVID and GO was used for pathways analysis. WGCNA was employed to identify discrete gene modules based on co-expressions between genes. Genotyping was generated on a custom-modified HumanCoreExome array, followed by imputation using Haplotype Reference Consortium (HRC) panel on Michigan Imputation Server.
Results: Pathway analysis of differentially expressed genes revealed stage-specific progression of AMD from healthy tissue to advanced stages. Several AMD associated genes, especially those associated with immune response, were up regulated in early stages with later alterations in complement and extracellular matrix pathways. WGCNA analysis identified several co-expression modules in normal and AMD retina. We are currently performing eQTL analysis for evaluating the impact of risk variants on gene regulation to gain novel insights into AMD.
Conclusions: Our data represent the largest resource of gene expression and its genetic regulation in vision research. Our findings highlight the genes, pathways and regulatory mechanisms involved in AMD. Additionally, it provides a useful resource for functional dissection of multiple other vision related traits.
Commercial Relationships: Rinki Ratnapriya, None; **Margaret R. Starostik**, None; **Rebecca Kapphahn**, None; **Ashley Walton**, None; **Alexandra Pietraszkiewicz**, None; **Matthew Brooks**, None; **Sandra R. Montezuma**, None; **Lars Fritsche**, None; **Goncalo Abecasis**, None; **Deborah A. Ferrington**, None; **Anand Swaroop**, None

Program Number: 4773
Presentation Time: 4:00 PM–4:15 PM
Effects of aging and chronic cigarette exposure to circulatory microRNAs in the Rhesus macaque
Zeljka Smit-McBride[1], Johnny Nguyen[1], Garrett W. Elliott[1], Anthony Nguyen[1], Christian Munevar[3], Glenn Yiu[1], Sara M. Thomasy[2], Kent E. Pinkerton[3, 4], Sharon L. Oltjen[1], Jeffrey Roberts[4], Lawrence S. Morse[1]. [1]Vitreo-Retinal Research Lab, Univ of California, Davis Sch of Med, Davis, CA; [2]Dept. of Surgical and Radiological Sciences, UC Davis Sch of Vet Med, Davis, CA; [3]University of California, Davis Sch of Vet Med, Davis, CA; [4]California National Primate Research Center, Davis, CA.
Purpose: AMD is a blinding disease having both genetic and environmental components, with cigarette smoke(CS) exposure as the leading environmental risk factor. The purpose of this study was to identify age-related circulatory microRNA(miRNA) changes in the plasma and ocular fluids of the Rhesus macaque and compare them to CS induced changes, with intent is to develop an animal model of dry AMD.
Methods: All Rhesus macaques were housed at the California National Primate Research Center(CNPRC) at UC Davis. We used 6 animals of the same gender per group: young (2-4 years old), old (20-25 years old) and middle aged (9-12 years old)-4 of which were exposed to smoke for 1 month. These 4 macaques were clinically assessed prior to and following CS exposure using Fourier-domain optical coherence tomography(FD-OCT) and fundus photography. Data was analyzed using a combination of Stata software and paired *t* tests. Ocular fluids and plasma samples were collected, miRNA isolated, and expression data obtained from Affymetrix miRNA GeneTitan Array Plates 4.0, followed by bioinformatics analysis on Affymetrix Expression Console(EC) and Transcriptome Analysis Software(TAS), and using ANOVA. Immunohistochemistry was performed on paraffin embedded ocular tissue sections.
Results: Retinal imaging using OCT, showed no significant changes in retinal thickness. Statistically significant changes were seen in miRNA populations in ocular fluids and plasma. In the plasma samples, 45 miRNAs were strongly upregulated (Fold Change >+/-1.5, $p<0.05$) upon CS exposure, while in aging monkeys a different miRNAs in plasma were affected, and downregulated. In the vitreous, 3 miRNAs were downregulated in animals exposed to CS, intriguing enough two of them (miR-6794 and miR-6790) were the same ones downregulated with age. Cotinine assays showed that animals received on average dose of 50-60 ng/ml cotinine, a marker for CS exposure. Immunohistochemistry of Sirt1, a marker of aging, showed decreased intensity of staining in retina with age as well as with CS exposure.
Conclusions: One month of CS exposure of Rhesus macaques resulted in significant changes of expression of circulatory miRNAs. Healthy aging changes of miRNAs populations were different from the CS exposure induced changes in plasma, while the ones in vitreous were similar. This data will be utilized to develop an animal model of dry AMD, using a monkey model exposed to CS.
Commercial Relationships: Zeljka Smit-McBride; **Johnny Nguyen**, None; **Garrett W. Elliott**, None; **Anthony Nguyen**, None; **Christian Munevar**, None; **Glenn Yiu**, Alcon (F), Carl Zeiss (C), Allergan (C); **Sara M. Thomasy**, None; **Kent E. Pinkerton**, None; **Sharon L. Oltjen**, None; **Jeffrey Roberts**, None; **Lawrence S. Morse**, Genentech (C)

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Presentation Time: 4:15 PM–4:30 PM
Long Range Genomic Interactions Regulate Photoreceptor Gene Expression
Philip A. Ruzycki[1, 2], Courtney D. Linne[1], Shiming Chen[1]. [1]Dept of Opthalmology and Visual Sciences, Washington University School of Medicine, St. Louis, MO; [2]Division of Biological & Biomedical Sciences, Washington University School of Medicine, St. Louis, MO.
Purpose: Precise packaging of genomic DNA into the nucleus is critical for assuring that the cell expresses certain genes and silences others. Photoreceptors offer a powerful system to determine the role of genomic DNA packaging. We recently discovered that, in rods, some highly expressed loci on the same or different chromosomes physically interact. The *Rhodopsin* (*Rho*) locus is the most active region of the rod genome and its mutation causes blinding diseases. This study aimed to determine whether inactivation of this locus affects genomic interactions and the expression of other genes in *trans*.
Methods: Long-range genomic interactions of the *Rho* locus in mouse retinas were identified using Circularized Chromatin Conformation Capture (4C) and confirmed using 3D-Fluorescence In Situ Hybridization (3DFISH) in retinas from wild-type *(WT)* mice and from other species. The functional importance of the *Rho* interactome was investigated in strains lacking Crx, Nrl, or with a CRISPR-mediated deletion of the *Rho* promoter or enhancer. 3DFISH, qRT-PCR, and RNAseq were used to reveal the consequences of these genetic manipulations on 3D genomic organization and expression of *Rho* and other genes with which *Rho* physically interacts.
Results: 4C and 3DFISH assays on P14 *WT* (rod-dominant) mouse retinas detected several long-range interactions of actively transcribed genes. In rods, *Rho* interacted specifically with some active regions on the same chromosome (*cis* interactions) and with several other rod-expressed genes on different chromosomes (*trans* interactions), but at lower frequencies. Retinal 3DFISH on different species suggests that these interactions are highly conserved, although they were lost in mice lacking Crx or Nrl. Mice lacking *Rho* promoter, but not its enhancer, also showed decreased *Rho* interactions. This decrease correlated with the loss of *Rho* transcripts and altered expression of *Rho*-interacting genes as detected by quantitative RT-PCR and RNAseq.
Conclusions: Together, our results suggest that *Rho* genomic interactions in the rod nucleus are important for co-expression of a subset of rod-specific genes. These interactions depend on cell type-specific transcription factors and *Rho* promoter activity. Inactivation of the *Rho* locus can affect other genes in *trans*. This previously unexplored epigenetic mechanism provides a new insight into pathogenic mis-regulation of gene expression in retina development and disease.
Commercial Relationships: Philip A. Ruzycki, None; **Courtney D. Linne**, None; **Shiming Chen**, None
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Program Number: 4775
Presentation Time: 4:30 PM–4:45 PM
Single cell RNA-seq provides insights into mechanisms driving photoreceptor development and degeneration
Melissa M. Liu[1], Ming-Wen Hu[1], Cynthia A. Berlinicke[1], Jiang Qian[1], Loyal Goff[2], Donald J. Zack[1]. [1]Wilmer Eye Institute, Johns Hopkins University School of Medicine, Baltimore, MD; [2]McKusick-Nathans Institute of Genetic Medicine, Johns Hopkins University School of Medicine, Baltimore, MD.
Purpose: Although photoreceptor gene expression has been studied in the contexts of normal postnatal development and rd1 degeneration using RNA-seq, the genetic drivers regulating these processes remain unknown. Kinetics studies of photoreceptor degeneration suggest that the trigger initiating cell death is stochastic, and signal from a stochastic driver is likely to be masked when pooling populations of cells, in which each individual cell is at a different stage on the path to degeneration. We hypothesize that these stochastic drivers of cell death may be better identified and characterized using single cell RNA-seq.
Methods: Fluorescence activated cell sorting was used to isolate single CD73+ rod photoreceptors from wildtype (wt) and rd1 mice. Smart-seq2 was used to prepare full length cDNA from single cells for Illumina sequencing library preparation. 94 single photoreceptors were sequenced from both wt and rd1 at postnatal days (P) 5, 8, 11, 14, and 28 (wt only), for a total of 846 single cells. A branched pseudotemporal trajectory representing photoreceptors undergoing normal development and rd1 degeneration was reconstructed using Monocle2. Candidate driver genes were identified via branched expression analysis modeling and experimentally tested using qPCR.
Results: Principal component analysis formed distinct clusters based on both age and strain. Pseudotemporal ordering reconstructed a trajectory in which all photoreceptors progressed together up to a distinct point, after which wt and rd1 photoreceptors diverged along two different trajectories. The wt trajectory recapitulated known features of normal postnatal photoreceptor development. The rd1 trajectory upregulated markers of cell stress and downregulated markers of photoreceptor maturity. The rd1 photoreceptors isolated from P5 and P8 also showed precocious development relative to their wt counterparts.
Conclusions: Single cell RNA-seq reconstructed highly resolved gene expression trajectories for photoreceptors undergoing normal postnatal development and rd1 degeneration, revealing novel insights into the genetic mechanisms driving these processes.
Commercial Relationships: Melissa M. Liu; **Ming-Wen Hu**, None; **Cynthia A. Berlinicke**, None; **Jiang Qian**, None; **Loyal Goff**, None; **Donald J. Zack**, None
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Program Number: 4776
Presentation Time: 4:45 PM–5:00 PM
In-depth genomic and lipidomic comparison of human and rabbit meibomian glands provides clues on the mechanisms of biosynthesis of meibum
Igor A. Butovich, Jadwiga C. Wojtowicz, Anne McMahon. Ophthalmology, University of Texas Southwestern Medical Center, Dallas, TX.
Purpose: Recently, we compared expression patterns of lipid metabolism-related genes in Meibomian glands (MG) of humans and mice, and linked them to their respective lipidomes. The gene expression profiles (GEP) and lipidomes of both species were found to be very similar. However, the complex metabolic pathway that leads to generation of meibum (which we call *meibogenesis*),

apparently, involves hundreds of proteins, whose roles are difficult to elucidate. To advance our knowledge of meibogenesis, we extended our studies into rabbits, who have meibum that is very different from the human one. By analyzing differentially expressed genes in MG of humans and rabbits, and correlating them with their corresponding lipid profiles, we attempted to identify genes and proteins that might be responsible for the biosynthesis of the major lipid components of meibum.

Methods: Specimens of tarsal plates of human subjects (n=4; HTP), mice (2 pooled samples, 6 animals each; MTP) and white rabbits (n=6; RTP) were collected and analyzed using mRNA microarrays, Expression and Transcriptome Analysis Consoles (from Affymetrix), and SigmaStat (Systat Software, Inc.). Samples of human and animal meibum were analyzed by gas and liquid chromatography and ion trap mass spectrometry.

Results: The intraspecies, intersubject variability of GEP in HTP, MTP, and RTP was low (about ±10%, or less, on a log2 scale, Figure 1), with occasional spikes in just a few out of ~300 genes analyzed. The intraspecies, intersubject variability in meibomian lipids (ML) did not exceed ±15% for major ML. However, when GEP in HTP and RTP were compared with each other (Figure 2), the interspecies differences became obvious. The RTP samples showed significantly higher levels of ELOVL1, TECR, FAR1, ACAT1, BCKDHB, ACADL, and LSS, among other genes that encode enzymes, transporters, and effectors, and much lower levels of SCD, ELOVL6, ACSL3, CERS6, ACADSB, and others, compared with HTP. The differences in GEP and ML were linked to a noticeably higher degree of saturation of ML, higher levels of lanosterol (LS) and dehydro-LS, and higher levels of diacylated diols in rabbit meibum, among other differences.

Conclusions: Genomic approaches, used in combination with metabolomic (lipidomic) ones, provided valuable information on the roles of specific enzymes in meibogenesis and other complex metabolic pathways.

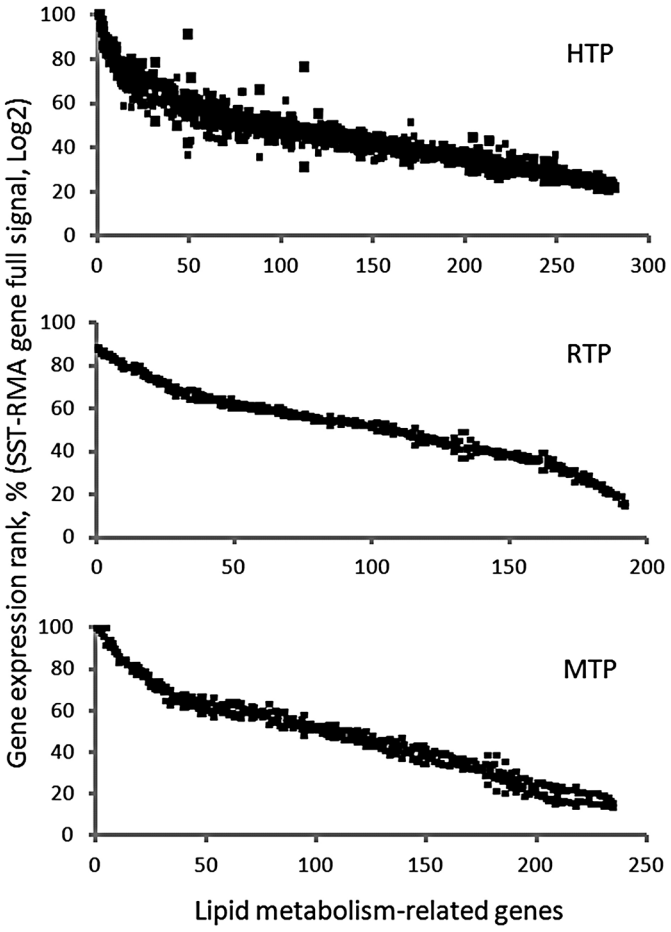

Fig. 1

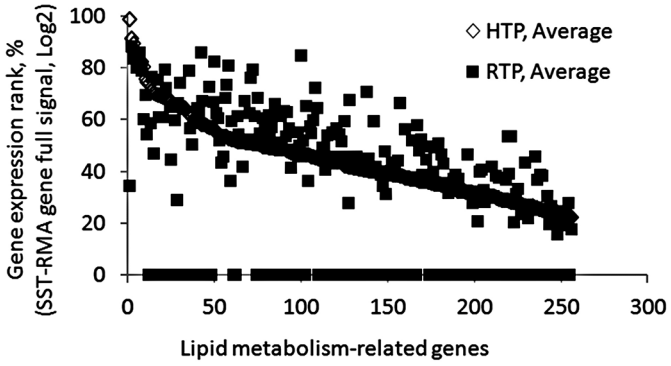

Fig. 2

Commercial Relationships: Igor A. Butovich, None; **Jadwiga C. Wojtowicz**, None; **Anne McMahon**, None

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Program Number: 4777
Presentation Time: 5:00 PM–5:15 PM
Developmental protein NUDC is crucial for rod maintenance and function
Alecia K. Gross[1], Evan R. Boitet[1], Guoxin Ying[2], Nicholas J. Reish[1], Wolfgang Baehr[2]. [1]Optometry and Vision Science, University of Alabama at Birmingham, Birmingham, AL; [2]Opthalmology and Vision Sciences, University of Utah, Salt Lake City, UT.
Purpose: Nuclear distribution protein C (NUDC) plays an essential role in mitosis and cytokinesis. Prior studies from our lab have shown NUDC interacts with rhodopsin and rab11a in rods. To determine if NUDC is critical for rod function, we have created transgenic *X. laevis* expressing either NUDC L280P or an shRNA directed against NUDC. Further, we generated a retina-specific *Nudc* knock-out mouse to identify its role in mouse retinal development.
Methods: Transgenic *X. laevis* tadpoles were prepared expressing mCherry-NUDC, NUDC L280P, or a bicistronic construct expressing EGFP and NUDC shRNA under the rod opsin promoter. Retina-specific *Nudc* knock-out mice were generated using $Nudc^{tm1a(KOMP)Mbp}$ embryonic stem cells obtained from the UCDavis KOMP repository and bred with Six3-Cre mice. For tadpole immunohistochemistry (IHC), eyes were fixed, cryosectioned and labeled with antibodies against rhodopsin, transducin, and arrestin. Cells were stained with wheat germ agglutinin, DAPI and TUNEL. Scotopic electroretinography (ERG) was performed on 7 week-old $Nudc^{-/-}$ mice. IHC was performed on retinal cryosections from these mice and labeled with DAPI and antibodies against rhodopsin, PDE6, cone arrestin, and PKCα.
Results: Expression of NUDC L280P or knock-down of *Nudc* causes slight mislocalization of rhodopsin and a strong staining pattern of rhodopsin at the base of the outer segments in *X. laevis*. Thionin staining of retina-specific *Nudc* knockout mouse whole retina sections revealed severe retinal degeneration involving all retina layers from photoreceptors to ganglion cells. Confocal IHC with DAPI, anti-rhodopsin and anti-PDE6 revealed reduced thickness of the mutant ONL, absence of OS, and presence of rhodopsin and PDE6 in the inner segments. However, rhodopsin and PDE did not mislocalize in the ONL. Labeling with anti-cone arrestin revealed the presence of cones lacking OS. The INL was reduced to 1-2 nuclear layers, and labeling with PKCα showed severe degeneration of rod bipolar cells. Ganglion cells are mostly absent. Scotopic ERG at 7 weeks was negative consistent with non-functional rods.
Conclusions: Here, we show NUDC has a critical role in rod cell maintenance, protein trafficking and homeostasis. It is essential for development and maintenance of all cells in the retina. The mouse knockout retina is severely degenerated, but rod, cone and bipolar cell remnants are present. The cause of degeneration is unknown.
Commercial Relationships: Alecia K. Gross; **Evan R. Boitet**, None; **Guoxin Ying**, None; **Nicholas J. Reish**, None; **Wolfgang Baehr**, None
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Program Number: 4778
Presentation Time: 5:15 PM–5:30 PM
Chronic activation of the unfolded protein response attenuates protein synthesis in the retinas of *rd16 mice*
Christopher R. Starr, Priyamvada M. Pitale, Marina Gorbatyuk. Optometry and Vision Sciences, University of Alabama at Birmingham, Birmingham, AL.
Purpose: In recent years, studies have demonstrated that chronic activation of the unfolded protein response (UPR) is involved in the pathogeneses of varied retinal dystrophies and plays a role in the activation of pro-inflammatory and aberrant calcium signaling that results in apoptotic photoreceptor (PR) cell death. The goal of this study is to test the hypothesis of whether a persistently activated UPR can negatively regulate protein synthesis in degenerating PRs.
Methods: Retinas were isolated from C57BL/6 (WT) and *rd16* mice at postnatal (p) day 15 and 20 to assess activation of the UPR by western blot analysis. To study the rate of protein synthesis, we used the surface sensing of translation (SUnSET) method. Mice were intraperitoneally injected with puromycin (Pu) (0.04μmol/g body weight), and after 30 min, retinas were harvested for western blot analysis using an anti-Pu antibody. Intensity of incorporated Pu was normalized to the total protein level detected by coomassie staining of the membrane. In addition, primary rod PRs were isolated from WT p20 mice. Primary PRs were treated with 3μM thapsigargin for 2h to activate the UPR followed by exposure to 20μM Pu for 30min. Levels of nascent protein synthesis were assessed by immunocytochemical analysis and quantitative fluorescence microscopy.
Results: The UPR markers PERK, BiP, ATF4, CHOP and ATF6 were significantly upregulated in *rd16* at p15 and p20; the elevation was in the range from 85% to 266%. Phospho-eIF2α, a hallmark of translational arrest, was elevated at p15 and p20 by 180 and 108%, respectively. In addition, phospho-4EBP1, whose level correlates with translation, was reduced by 30% at both p15 and p20. These levels were associated with a 26% reduction in protein synthesis at p15 as detected by the SUnSET technique. Results of the in vivo study were confirmed by experiments with Pu-treated primary PRs demonstrated that UPR activation inhibits the rate of newly synthesized proteins by over 3-fold.
Conclusions: *Rd16* retinas with a persistently activated UPR undergo translational inhibition. Future experiments may shed light on the role of impaired protein synthesis in the function of degenerating PRs. Strategies that reduce the duration of the PERK-phospho-eIF2α branch and restore protein synthesis could be applied as a novel therapeutic approach to retard retinal degeneration.
Commercial Relationships: Christopher R. Starr, None; **Priyamvada M. Pitale**, None; **Marina Gorbatyuk**, None