271 Molecular and biochemical mechanisms in retinal disorders
Monday, May 08, 2017 3:45 PM–5:30 PM
Room 314 Paper Session
Program #/Board # Range: 2050–2056
Organizing Section: Biochemistry/Molecular Biology

Program Number: 2050
Presentation Time: 3:45 PM–4:00 PM
Rapid, high throughput vision analysis of candidate genes in zebrafish
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Purpose: Modern genomics has enabled the identification of an unprecedented number of genetic variants, many of which are extremely rare, associated with blinding disorders. These advances underscore the need for tractable models to rapidly validate candidates as disease causing and to characterize their functional roles. Zebrafish eye structure is similar to humans and the rapid development and small size of zebrafish facilitates genetic screens. In addition, zebrafish display robust quantifiable behavioral responses to visual stimuli. The goal is to evaluate a spectrum of visual impairments to facilitate high-throughput in vivo screens.
Methods: Our lab uses two specific assays in five day old zebrafish: Visual Interrogation of Zebrafish maNipulations (VIZN) and OptoMotor Response (OMR). VIZN uses interruptions in constant light to induce a startle response. Fish that are visually compromised will not respond to the interruption of light. We simultaneously capture the motion of 96 larval zebrafish in 1 second intervals as we subject them to "startle" stimuli with ViewPoint hardware. We developed software (VIZN) in the R programming language to automatically analyze the response of individual zebrafish at each stimulus, group by genotype, plot the data, and perform statistics. In OMR, fish move in the direction of apparent motion created by an animated gradient of sinusoidal waves. Larvae respond by orienting their body and swim the direction of the motion, while the visually impaired move in random directions. To quantify behavior, we developed a scoring rubric.
Results: We have successfully used VIZN to validate candidate vision loss genes. Blind fish also do not respond to OMR. In preliminary analysis, activity of selected cataract and glaucoma genes were manipulated in zebrafish and we find that they are responsive to VIZN, but not to OMR. We are currently adapting the OMR into a high throughput test for partial vision loss. To understand the functional roles, we evaluate retinal structure and expression domains in the developing zebrafish.
Conclusions: These assays allow for rapid screening of many candidate genes related to vision loss and hold the potential to follow disease progression and serve as a platform for drug testing.
Commercial Relationships: Diane C. Slusarski, None
Support: NIH, R01 EY011298

Program Number: 2051
Presentation Time: 4:00 PM–4:15 PM
Deciphering the molecular network surrounding ZNF408 in the regulation of retinal angiogenesis
Dyah W. Karjosukarso[1, 2], Sebastianus H. van Gestel[1, 3], Jieqiong Qu[1, 3], Evelyn N. Kouwenhoven[1, 3], Lonneke Duijkers[1, 2], Huiqing Zhou[1, 3], Rob W. Collin[1, 2]. [1]Human Genetics, Radboudumc, Nijmegen, Netherlands; [2]Donders Institute for Brain, Cognition, and Behaviour, Nijmegen, Netherlands; [3]Molecular Developmental Biology, Faculty of Science, Radboud University, Nijmegen, Netherlands.
Purpose: A missense mutation in *ZNF408* (p.His455Tyr) was reported in a family with an autosomal dominant form of familial exudative vitreoretinopathy (FEVR). *ZNF408* encodes a protein that belongs to the zinc finger transcription factor family. Previous *in vitro* and *in vivo* studies implied the pathogenicity of the mutation and revealed an important role for ZNF408 in the development of vasculature. Nonetheless, little is known about the downstream transcriptional targets of ZNF408 and how it contributes to (retinal) angiogenesis.
Methods: The molecular mechanisms of *ZNF408*-associated FEVR were investigated *in vitro* by overexpression of wild-type and mutant ZNF408 in cellular systems. Chromatin immunoprecipitation followed by next generation sequencing (ChIP-Seq) was employed to reveal the genome-wide ZNF408 binding sites. Subsequently, RNA sequencing (RNA-Seq) in a vascular endothelial cell line was performed to determine the genes whose expression is dependent on ZNF408.
Results: The data obtained by ChIP-SEQ showed that ZNF408 binds predominantly to promoter regions of genes involved in various processes, including, but not limited to, protein localisation, macromolecular complex assembly, and RNA processing. Interestingly, the data obtained also showed that the mutant ZNF408 has a reduced ability to bind to its targets. These findings were validated by luciferase-based transactivation assays on a selected subset of targets. Transcriptome analysis revealed distinct gene expression profiles in cellular models overexpressing wild-type ZNF408 compared to mutant ZNF408. Furthermore, the transcriptome data allowed the identification of genes whose expression is dependent on ZNF408.
Conclusions: The combination of the ChIP-Seq and RNA-Seq datasets allowed the identification of the transcriptional targets of ZNF408. It also allowed us to clearly distinguish the differential role of wild-type versus mutant ZNF408 in the regulation of transcription in the context of (retinal) angiogenesis. Finally, the identification of the transcriptional targets of ZNF408 may reveal novel candidate genes for unsolved FEVR cases and thereby increase our understanding on the molecular mechanisms underlying FEVR.
Commercial Relationships: Dyah W. Karjosukarso, None; **Sebastianus H. van Gestel**, None; **Jieqiong Qu**, None; **Evelyn N. Kouwenhoven**, None; **Lonneke Duijkers**, None; **Huiqing Zhou**, None; **Rob W. Collin**, None

Program Number: 2052
Presentation Time: 4:15 PM–4:30 PM
Bardet-Biedl syndrome-8 (BBS8) is essential for morphogenesis of photoreceptor outer segments
Tanya Dilan[1], Ratnesh Singh[2], Thamaraiselvi Saravanan[2], Andrew F. Goldberg[3], Visvanathan Ramamurthy[2, 1]. [1]Biochemistry, West Virginia University, Morgantown, WV; [2]Eye Institute, West Virginia University, Morgantown, WV; [3]Eye Research Institute, Oakland University, Rochester, MI.
Purpose: Bardet-Biedl Syndrome complex (BBSome) is a multiprotein complex linked to retinitis pigmentosa, yet its role in photoreceptors remains poorly understood. It is thought that the BBSome is important for ciliogenesis and it has also been linked to anterograde protein trafficking. However, recent studies have questioned these findings in photoreceptors. The purpose of this study is to tease out the molecular mechanisms underlying the photoreceptor pathology in Bardet-Biedl Syndrome. We have generated multiple animal models lacking BBS8, a core component of the BBSome, focusing on identifying the role of BBS8 in vision.
Methods: To study the role of BBS8/BBSome in photoreceptors, we generated four different animal models:1)A global knockout,

2)Retina-specific conditional, 3)Cone-photoreceptor specific conditional and 4)Lastly, to investigate the role of BBS8 in maintenance of photoreceptors, a rod-specific inducible knockout was generated. Retinal morphology was examined by light- and electron microscopy. Protein expression and subcellular localization were analyzed by immunoblotting and immunocytochemistry, respectively.
Results: Ultrastructural analysis of our global knockout mice revealed dysmorphic photoreceptor outer segments at early stages of photoreceptor development (postnatal day 10-P10). Interestingly, at P10, exo-vesicles were abundant in the extracellular space. Cone-specific model revealed the need for BBS8 in survival and function of cone photoreceptors. Additionally, in the absence of BBS8, we observed dynamic changes of other BBS partner subunits. Ablation of BBS8 led to the accumulation of Syntaxin3 (STX3), a protein normally found in the IS and synapse, in the rod OS at P10. Interestingly, cone-specific deletion of BBS8 led to a similar accumulation of STX3 in the cone OS, prior to defects in cone function (Fig 1).
Conclusions: Our results show the need for BBS8 in development of photoreceptor outer segments. In comparison to other animal models for BBS, removal of BBS8 results in early and severe dysmorphogenesis of the OS. It is not clear if the observed accumulation of STX3 contributes to the photoreceptor pathology and/or is the byproduct of defective photoreceptor morphology. Our current efforts are focused on distinguishing between these two mechanisms with the use of inducible animal models.

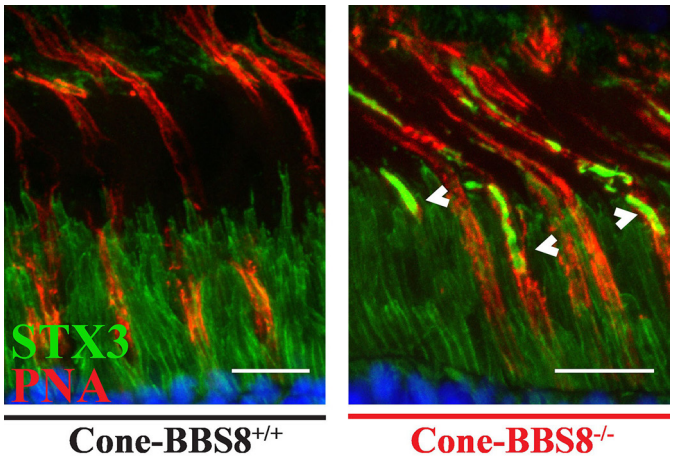

Figure 1:Accumulation of Syntaxin 3 in cone photoreceptors.Scale bar: 10 microns.
Commercial Relationships: Tanya Dilan, None; **Ratnesh Singh**, None; **Thamaraiselvi Saravanan**, None; **Andrew F. Goldberg**, None; **Visvanathan Ramamurthy**, None
Support: R01EY025536,R01EY017035

Program Number: 2053
Presentation Time: 4:30 PM–4:45 PM
Congenital knock-out of transition zone protein BBS5 reveals cone-rod dystrophy with protein mislocalization
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Purpose: The BBSome is a stable network of eight proteins that has been found necessary for normal cilia function and is involved in vesicular trafficking to the ciliary membrane. Mutations occurring in BBSome components result in retinal degeneration-associated ciliopathies. In photoreceptors the precise role of BBSome remains elusive and the function of the protein Bardet-Biedl Syndrome 5 (BBS5) is currently unknown.
Methods: BBS5 knock-out (KO) mice were generated with ES cells from Eucomm. Electroretinography (ERG) scotopic and photopic tests were performed on 2 month-, 4 month-, 6 month- and 9 month-old mice using the HMsERG LAB system from OcuScience. For immunohistochemistry (IHC), eyes were enucleated, fixed in 4% paraformaldehyde, embedded and cut into 12 micron sections. Sections were then labeled with antibodies against rhodopsin (B6-30N), transducin (SC-389, Santa Cruz), visual arrestin (SC-67130, Santa Cruz), and stained for wheat germ agglutinin (WGA) and nuclei (DAPI). TUNEL staining was performed to analyze cell death. Transmission electron microscopy (TEM) was performed on 3 month-old animals.
Results: At 2 months of age, mouse ERG responses were completely diminished in BBS5 KO mice, while scotopic responses were reduced. IHC revealed mislocalization of rhodopsin, arrestin and transducin. Labeling with WGA showed that outer segments are shortened significantly by 2 months, while interestingly there is only a reduction of 2 rows of nuclei in the outer nuclear layer by 9 months. TUNEL staining revealed a significant increase of cell death by two months. TEM showed the appearance of mostly normal disks with very few areas of abnormal membranes in BBS5 KO photoreceptors.
Conclusions: Based on these results we find BBS5 plays a role in photoreceptor function and outer segment protein localization. By 2 months of age, BBS5 KO mice lose cone function entirely, accompanied by loss of rod function via ERG analyses. Severe mislocalization of rhodopsin, arrestin and transducin are found as well. TEM reveals the appearance of mostly normal disk formation. These data support the hypothesis that BBS5 plays a vital functional role within photoreceptors and protein trafficking.
Commercial Relationships: Katie Bales, None; **Mandy J. Croyle**, None; **Bradley K. Yoder**, None; **Alecia K. Gross**, None
Support: NIH NEI EY019311, the EyeSight Foundation of Alabama, Karl Kirchgessner Foundation and the E. Matilda Ziegler Foundation.

Program Number: 2054
Presentation Time: 4:45 PM–5:00 PM
Upregulation of myopia-related genes in the Interphotoreceptor retinoid-binding protein (IRBP) knockout (KO) mouse model
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Purpose: Untreated myopia, a refractive error disorder is the most common cause of visual impairment worldwide and is associated with heavy economic burden. Mechanisms underlying myopia are not yet clear. One of the primary focuses of our laboratory is to understand the role of IRBP in normal eye development. IRBP is a major protein of the sub-retinal space and plays a crucial role in the visual cycle. Mutations in human RPB3 (IRBP) gene are associated with high myopia and retinal dystrophy. We previously reported eye size defects, (Post-natal day, P8), profound myopia and retinal degeneration (RD), at P23 in the IRBP KO mice. The purpose of this study was to test the hypothesis that several genes implicated in myopia are upregulated in the retinas of IRBP KO mice at P5, an age preceding myopia in IRBP KO mice and at P213, post RD.
Methods: Retinal cDNA from C57BL/6J (WT) and congenic IRBP KO mice at P5 (WT, n=8; KO, n=5) and P213 (WT, n=8; KO, n=6) were subjected to digital droplet PCR. A total of 28 genes associated with myopia were selected. HPRT was used as a housekeeping gene. Ratio (target/HPRT), standard error of the mean (SEM) were

recorded. An unpaired t-test with Welch's correction assuming unequal variances was used to assess statistical significance. A *p* value <0.05 was considered significant.
Results: Data analysis revealed upregulation in several genes in IRBP KO mice. Interestingly, most of the altered genes at P5 were not significantly different at P213 and *vice versa*. At P5, *Aplp2*, *Calb1*, *Gjd2, Isl1*, *Sntb1* (p<0.01); *Vsx2*, *Ripk2* and *Egr1* (p<0.05) were upregulated while *Tox* (p<0.01) was downregulated. At P213, *C1q*, *Grm6, Isl1 and Otx2* (p<0.01); *Calb2*, *Sntb1*and *Zeb2* (p<0.05); *Vsx2* and *Tox* (p<0.001) were upregulated.
Conclusions: Our data support the hypothesis that several of myopia-genes are upregulated in IRBP KO retinas indicating the importance of IRBP in early eye size determination and in myopia pathogenesis. Our results highlight the importance of *Aplp2*, *Sntb1*, *Isl1*, *Tox* and *Vsx2* signaling pathways and suggest targets to treat IRBP mediated myopia and RP. This may suggest genetic mechanisms in the predilection to increased axial length before vision or light mediated signaling.
Commercial Relationships: Shanu Markand; **Micah A. Chrenek**, None; **Priyanka Priyadarshani**, None; **Jeffrey H. Boatright**, None; **J M. Nickerson**, None
Support: Abraham J. & Phyllis Katz Foundation, NIH R01EY014026, R01EY021592, R01EY016470, VA RR&D C1924P I21RX001924, VA RR&D C9246C (Atlanta VA Center of Excellence in Vision and Neurocognitive Rehabilitation), P30EY006360, and an unrestricted grant to the Department of Ophthalmology at Emory University from Research to Prevent Blindness, Inc.

Program Number: 2055
Presentation Time: 5:00 PM–5:15 PM
De-regulated cilia and UPS genes among canine *RPGRIP1* mutants with absent cone ERG
Gautami Das[1], Matthew Brooks[2], Alexis Boleda[2], Anand Swaroop[2], Gustavo D. Aguirre[1], Keiko Miyadera[1]. [1]School of Vet Medicine, University of Pennsylvania, Philadelphia, PA; [2]National Eye Institute, NIH, Bethesda, MD.
Purpose: Although defect in cilia gene, *RPGRIP1* causes cone-rod dystrophy1 in dogs (cord1), significant discordance in *RPGRIP1*$^{ins/ins}$ genotype and phenotype exists among dog breeds. In a colony of *RPGRIP1*$^{ins/ins}$ mutants none has clinical blindness for over 8 years. However, their cone-derived electroretinograms (ERG) range from normal, reduced to absent. To tease out the basis for the phenotype continuum under a specific genetic background we studied structurally and transcriptomically retinas of mutants with normal- and absent-cone ERG.
Methods: Retinal sections were studied by H&E and immunohistochemistry (IHC). Additionally, Illumina GAIIx 76bp single-end RNA-seq was performed as a pilot study from retinal tissues of *RPGRIP1*$^{ins/ins}$ dogs with normal (n=2, 9-11 months) or absent (n=2, 9 months) cone ERGs. This was followed by total transcriptomics analysis in wild type (2F, 2M, 6-29 mon), *RPGRIP1*$^{ins/ins}$ with normal- (1F, 3M, 5-9 mon) and absent- (2F, 2M, 7-9 mon) cone ERG samples and sequenced (150x2 bp) in Illumina Next seq.To validate expression of selected genes qRT-PCR was performed.
Results: Absent-cone ERG retina had truncated cones with short outer and inner segments, significantly shorter ciliary axoneme and transition zone (p<0.001). None of the cone expressed transcripts showed significant differential expression (DE) although IHC indicated mislocalization, faint labeling or absence of some cone transduction proteins. However, RPKM and ct values were comparable. Of the 55 DE transcripts identified by RNA-seq (1.5<fold change>-1.5) following multiple correction, genes involved in retinal diseases were down regulated whereas those regulating molecular trafficking, transcription and/or expressed in cilia, cellular structure etc. were up regulated. Although DE genes identified by total transcriptome study were not same many had function similar to those identified by RNA-seq. Interestingly, several disease causing cilia genes including *BBS9*, *RPGR*, *SPATA7* etc. were down regulated while ubiquitin proteasomal system (UPS) was up regulated.
Conclusions: A balance between positive and negative regulators of ciliogenesis is important to maintain cilia structure that is de-regulated in absent-cone ERG dogs.
Commercial Relationships: Gautami Das, None; **Matthew Brooks**; **Alexis Boleda**, None; **Anand Swaroop**, None; **Gustavo D. Aguirre**, None; **Keiko Miyadera**, None
Support: EY-06855 and -017549; Foundation Fighting Blindness; Macula Vision Research Foundation; Hope for Vision; Van Sloan Foundation.

Program Number: 2056
Presentation Time: 5:15 PM–5:30 PM
ARL3-GTP and its GEF ARL13b regulate photoreceptor transition zone formation
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Purpose: Arf-like Protein 3 (ARL3) is a ubiquitous small GTPase--the activity of which is regulated by its GAP (RP2 protein) and GEF (ARL13b). Mutations affecting the ARL13b G-domain lead to Joubert Syndrome in human, affecting primary cilia of kidney, brain, liver and other organs. We probed the function of ARL13b in mouse photoreceptors by generating a retina-specific (prefix ^{ret}) and tamoxifen-induced (prefix ^{tam}) *Arl13b* knockouts.
Methods: *Arl13b*$^{flox/flox}$ mice (floxed exon 2) were provided by Tamara Caspary (Emory). Retina-specific knockout mice were obtained by mating *Arl13b*$^{flox/flox}$ mice with Six3Cre^{+} transgenic mice and adult tamoxifen-induced knockout mice by mating with EtCre^{+} transgenic mice. Mutant mice were crossed with CETN2-EGFP transgenic mice to identify photoreceptor centrioles and connecting cilia. Photoreceptor function was measured by ERG and OptoMotry; progress of retina degeneration was evaluated by optical coherence tomography, confocal immunohistochemistry, histology and TEM.
Results: ARL3-GDP distributes within the inner segment as a soluble protein while its GEF, ARL13b, localizes to the outer segment exclusively. The distinct localizations indicate that ARL3-GTP likely forms in the transition zone as a gradient. In P10 ^{ret}*Arl13b*$^{-/-}$ retina, photoreceptor connecting cilia were absent centrally as indicated by the transition zone marker, CETN2-EGFP, and the retina phenotype resembles LCA. Ultrastructure performed at P6 and P10 revealed that basal bodies docked to the photoreceptor cortex but transition zones were stunted in length and outer segments were not formed. As a result, outer segment proteins mislocalized in the inner segment and ONL. In ^{tam}*Arl13b*$^{-/-}$ retina at 2 weeks post-injection, ARL13b is completely depleted resulting in mislocalization of phototransduction proteins and destabilization of the axoneme.
Conclusions: Based on Joubert syndrome-like phenotypes of germline Arl3 and Arl13b knockouts, ARL3-GTP is key for ciliogenesis and transition zone formation in photoreceptors, as well as in primary cilia of other tissues affected by ARL13B null alleles in human.

Commercial Relationships: Wolfgang Baehr, None; **Christin Hanke-Gogokhia**, None; **Houbin Zhang**, None; **Jeanne M. Frederick**, None
Support: EY08123-28; P30 EY014800; EY019298-08; RPB NELSON TRUST; RPB unlimited grant to the Department of Ophthalmology U of Utah