507 Photoreceptor disease and rescue
Thursday, May 07, 2015 8:30 AM–10:15 AM
Exhibit Hall Poster Session
Program #/Board # Range: 5387–5412/A0236–A0261
Organizing Section: Retinal Cell Biology
Contributing Section(s): Biochemistry/Molecular Biology, Genetics, Physiology/Pharmacology, Retina, Visual Neuroscience

Program Number: 5387 **Poster Board Number:** A0236
Presentation Time: 8:30 AM–10:15 AM
Roles of Histone H3K27 tri-methylation for maintenance of rod photoreceptors
Sumiko Watanabe[1], Toshiro Iwagawa[1], Akira Murakami[2]. [1]Molecular & Developmental Biol, Univ of Tokyo, Inst Med Science, Tokyo, Japan; [2]Department of Ophthalmology, Juntendo University Graduate School of Medicine, Tokyo, Japan.
Purpose: Cell differentiation and maintenance are regulated by transcription factor- as well as epigenetic-network. We have been analyzing the roles of Histone H3K27 tri-methylation (H3K27me3) for retinal development using retina specific knockout mice of Ezh2, which is a H3K27 methyltransferase. We found that outer nuclear layer (ONL) became much thinner in Ezh2-CKO retina after birth, suggesting that H3K27me3 participates for maintenance of rod photoreceptors. In this report, we aimed to reveal more detailed molecular signature and mechanisms of roles of H3K27me3 for maintenance of rod photoreceptors.
Methods: To compare molecular signature of rod photoreceptor and other cell types, we purified rod and other cells by using a cell sorter in terms of expression of Cd73, which is a specific cell surface marker of rod photoreceptors, from developing mouse retina. Using purified Cd73 positive (Cd73P) and negative (Cd73N) cell fractions, we performed RNAseq and ChIPseq for H3K4me3 and H3K27me3. Roles of Ezh1/2 and Jmjd3 for ONL maintenance were examined by loss- and gain-of-function analyses.
Results: By bioinformatics approach using RNAseq data of Cd73P and Cd73N cells at P1, P5, P8, we selected genes specifically expressed either is rod photoreceptors (Cd73P-genes) or other cell types (Cd73N-genes). Analysis of ChIP-seq data suggested that H3K27me3 modification at loci of Cd73P-genes was observed neither in Cd73P and Cd73N cell fractions. In contrast, H3K27 at loci of Cd73N-genes was tri-methylated, and comparison between Cd73P and Cd73N fractions indicated that the Cd73N-gene-loci were H3K27 tri-methlated much stronger in Cd73P cells than in Cd73N cells. Immunostaining of rhodopsin and S-opsin of retinas derived from Ezh2-CKO or Ezh1-KO mice suggested that rod was degenerated but cone was relatively intact in the retinas of these mice.
Conclusions: Rod photoreceptor specific H3K27me3 modification in loci of genes, which are not expressed in rod, was observed. Since loss of function of Ezh2 or Ezh1 resulted in failure of proper maintenance of rod, we hypothesized that suppression of transcriptional activation of genes, which are unnecessary to maintain rod, by H3K27 tri-methylation in their loci is critical to prevent rod photoreceptors from degeneration.
Commercial Relationships: Sumiko Watanabe, None; **Toshiro Iwagawa**, None; **Akira Murakami**, None
Support: a grant-in-aid from the Ministry of Education, Culture, Sports, Science, and Technology of Japan

Program Number: 5388 **Poster Board Number:** A0237
Presentation Time: 8:30 AM–10:15 AM
Identification of FERM and PDZ domain containing 1 (*Frmpd1*) as a candidate gene necessary for rod photoreceptor maturation
Christie K. Campla[1, 2], Jung-Woong Kim[1], Hyun-Jin Yang[1], Anand Swaroop[1]. [1]Neurobiology Neurodegeneration & Repair Laboratory, National Eye Institute, Bethesda, MD; [2]Nuffield Laboratory of Ophthalmology, University of Oxford, Oxford, United Kingdom.
Purpose: The differentiation of rod photoreceptors is stringently regulated by a number of transcription factors, which include NRL (neural retina leucine zipper) and CRX (cone-rod homeobox). We hypothesized that genes dramatically increasing in expression during rod photoreceptor development and regulated by NRL and CRX would play a substantial role in the functional maturation of rods, and that cellular adhesion genes involved in this process would be important for establishing and stabilizing proper organization of photoreceptors within the outer nuclear layer.
Methods: RNA-seq data from FACS-sorted murine rod photoreceptors (from transgenic mice expressing GFP under the control of the *Nrl* promoter) was used to select for candidate cell adhesion genes differentially upregulated in rods from P2-P28. The rod-enriched protein expression of one such gene, *Frmpd1*, was confirmed by Western immunoblotting. To determine the effect on retina morphology upon *Frmpd1* knockdown, a variety of commercially available shRNA constructs were used to transfect murine retinal cells at P1 via *in vivo* electroporation. Eyes were harvested at various ages (P7, P10, P15, P21) and processed for immunohistochemistry to assess morphological consequences of shRNA-mediated *Frmpd1* knockdown.
Results: There was a noticeable decrease in *Frmpd1* expression in the $Nrl^{-/-}$ cone-dominated retina and enrichment in the rod-dominated $Nrl^{+/+}$ retina at both the RNA and protein levels. ShRNA-mediated knockdown of *Frmpd1* resulted in severe disruption of retina organization and photoreceptor morphology. Many of the *Frmpd1* knockdown photoreceptors were abnormally distributed within the inner and outer nuclear layers and appeared to have shortened outer segments with reduced expression of rhodopsin; in some cases, the outer segments clustered together to form "puckered" regions and rosettes in the outer nuclear layer.
Conclusions: *Frmpd1* is likely to play a substantial role in the functional maturation of rods, and particularly in establishing and stabilizing proper organization of photoreceptors within the outer nuclear layer.
Commercial Relationships: Christie K. Campla, None; **Jung-Woong Kim**, None; **Hyun-Jin Yang**, None; **Anand Swaroop**, None

Program Number: 5389 **Poster Board Number:** A0238
Presentation Time: 8:30 AM–10:15 AM
PROGRESS TOWARDS GENERATING ZEBRAFISH MODELS OF RETINITIS PIGMENTOSA
Hannah E. Henson, Marie A. Forbes-Osborne, Sara N. Perkins, Ann C. Morris. Biology, University of Kentucky, Lexington, KY.
Purpose: The inherited disease retinitis pigmentosa (RP) causes progressive degeneration of photoreceptors with a number of cases due to mutations in rhodopsin (RHO). Zebrafish provide an attractive model for retinal diseases because they possess a cone-rich retina. Here, we describe progress towards generating constitutive and inducible zebrafish lines expressing mutant RHO alleles previously identified in patients with RP, thereby allowing us to study the genotype-phenotype relationship in photoreceptor degeneration. Also, because zebrafish regenerate photoreceptors, we can discover pathways promoting retinal regeneration, which may suggest novel approaches to induce regeneration in mammals.

Methods: For constitutive lines, mutations in human RHO included P23H, R135L, and K296E. Using the Tol2 kit, mutant RHOs were subcloned from pGEM®-T into pME-MCS using pME-mCherry as a control. We also subcloned the *Xenopus* rhodopsin promoter (XOPS5.5) into the p5E-MCS vector. Using LR cloning, p5E(XOPS5.5), pME(RHOs or mCherry), and p3E(polyA) were inserted into the pDestTol2CG2 vector. To confirm constitutive constructs were functional, we injected one- to two-cell stage embryos with 30 pg of pDestTol2CG2(XOPS5.5:mCherry:polyA) DNA or 30pg DNA + 30 pg transposase RNA. Embryos were screened for mCherry expression in rods. Inducible lines were generated using the Tet-ON system and a biTRE-mCherry vector. Mutant RHOs were subcloned into pME-MCS. The p5E(dA-mCherry:biTRE), pME(RHOs), and p3E(polyA) were inserted into the pDestTol2CG2 vector.
Results: We successfully generated constructs for P23H, R135L and K296E constitutive lines, along with mCherry. For embryos injected with pDestTol2CG2(XOPS5.5:mCherry:polyA) DNA, 41% survived past 3 dpf of which 32% had mCherry+ rods. For those co-injected with DNA + transposase, 33% survived past 3dpf and 20% of those had mCherry+ rods. For inducible lines, we successfully generated constructs for the P23H, R135L, and K296E mutations.
Conclusions: We confirmed that XOPS5.5 drives expression of mCherry in rods. We also generated constructs expressing mutations in human RHO and inducible constructs co-expressing mutant RHO and mCherry. Future experiments will generate stable transgenic lines expressing mutant RHOs. The inducible lines will enable us to induce expression at various developmental stages to study degeneration, and turn off expression to study retinal regeneration.
Commercial Relationships: Hannah E. Henson, None; **Marie A. Forbes-Osborne**, None; **Sara N. Perkins**, None; **Ann C. Morris**, None

Program Number: 5390 **Poster Board Number:** A0239
Presentation Time: 8:30 AM–10:15 AM
Histone deacetylase 1 (HDAC1) is essential for rod differentiation in the mouse retina
Renata Carmona e Ferreira, Evgenya Popova, Daniel Hass, Jessica James, Samuel S. Zhang, Colin J. Barnstable. Neural and Behaviour Science, Pennsylvania State University, Hershey, PA.
Purpose: Previous studies have linked nonselective inhibition of all histone deacetylases using Trichostatin A or Sodium Butyrate to a complete blockage of rod photoreceptor development. These studies, however, fail to narrow down the specific HDACs important in retinal development. Here we investigate two HDAC classes possibly involved in rod photoreceptor growth in neonatal mice retinas. Our hypothesis is that histone deacetylation is essential for proper retina development and that inhibition of one specific HDAC will block rod photoreceptor differentiation.
Methods: Animal use was in accordance to ARVO/IACUC guidelines. Postnatal day 1 mice retinas were dissected from C57Bl6 mice and cultured for 96 hours with or without treatments of HDAC inhibitors Sodium Butyrate, MC1568, entinostat, apicidin and CAS 193551-00-7. They were then fixed in 4% paraformaldehyde, equilibrated in 20% sucrose, embedded and cryosectioned. Immunohistochemical staining was performed using antibodies for rhodopsin, H3K9ac, H4K12ac, PCNA and recoverin proteins. Image quantifications were done using Image J.
Results: Select inhibition of Class IIa HDACs using MC1568 at differing concentrations resulted in little to no change of rhodopsin expression or the level of H3K9ac. On the other hand, inhibition of Class I HDACs, HDAC1 and HDAC3, using entinostat resulted in a loss of retina structure comparable to that of nonspecific HDAC inhibition. A significant increase in H3K9ac and H4K12ac levels and a decrease in rhodopsin expression were observed. The selective inhibitors CAS 193551-00-7 (HDAC1) and apicidin (HDAC3) were then used to investigate which HDAC, 1 or 3 or both, have a role in rod development. No statistically significant change in rhodopsin expression and acetylation levels were found in retinas treated with apicidin. However, when retinas were treated with CAS 193551-00-7 a statistically significant decrease in rhodopsin expression accompanied with a statistically significant increase in histone acetylation levels were observed.
Conclusions: These findings suggest that the Class I HDAC1 is essential in regulating the expression of critical genes required for neonatal rod photoreceptor development.
Commercial Relationships: Renata Carmona e Ferreira, None; **Evgenya Popova**, None; **Daniel Hass**, None; **Jessica James**, None; **Samuel S. Zhang**, None; **Colin J. Barnstable**, None

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Presentation Time: 8:30 AM–10:15 AM
Genotypic and Phenotypic Characterization of the P23H Line 1 Rat Model
Elise Orhan[1], Deniz Dalkara[1], Christophe Lechauve[1], Serge A. Picaud[1], Thierry D. Leveillard[1], Jose A. Sahel[2, 3], Muna I. Naash[4], Matthew M. LaVail[5, 6], Christina Zeitz[1], Isabelle S. Audo[7, 3]. [1]INSERM U968, CNRS UMR_7210, Sorbonne Universités UPMC Univ Paris 06 UMR_S 968, Institut de la Vision, Paris, France; [2]INSERM U968, CNRS UMR_7210, Sorbonne Universités UPMC Univ Paris 06 UMR_S 968, Centre Hospitalier National d'Ophtalmologie des Quinze-Vingts DHU ViewMaintain, Fondation Ophtalmologique Adolphe de Rothschild, Academie des Sciences, Paris, France; [3]Institute of Ophthalmology University College of London, London, United Kingdom; [4]Department of Cell Biology, University of Oklahoma Health Sciences Center, Oklahoma City, OK; [5]Department of Ophthalmology, University of California San Francisco, San Fransisco, CA; [6]Department of Anatomy, University of California San Francisco, San Fransisco, CA; [7]INSERM U968, CNRS UMR_7210, Sorbonne Universités UPMC Univ Paris 06 UMR_S 968, Centre Hospitalier National d'Ophtalmologie des Quinze-Vingts DHU ViewMaintain, Paris, France.
Purpose: Rod-cone dystrophy, a member of the retinitis pigmentosa (RP) family of diseases is the most common inherited degenerative photoreceptor disease, for which no therapy is currently available. The P23H rat is one of the most commonly used autosomal dominant RP models. It has been created by incorporation of a mutated mouse rhodopsin (*Rho*) transgene in the wild-type (WT) Sprague Dawley rat. Detailed genetic characterization of this transgenic animal has never been fully documented. The current study proposes to fill this gap of knowledge on the P23H Line 1 (P23H-1) rat and provide additional phenotypic information applying non-invasive and state-of-the-art *in vivo* techniques that are relevant for preclinical therapeutic evaluations.
Methods: Transgene sequence was identified by Sanger sequencing. Using quantitative PCR (qPCR), transgene copy number was calculated and expression measured. Visual function of 1-, 2-, 3-, 6- and 7-months-old WT and P23H-1 rats was measured by full field electroretinography (ERG). Retinal structure was monitored using spectral domain optical coherence tomography (SD-OCT).
Results: Transgene sequencing reveals a P23H mutated mouse *Rho* sequence encompassing from the promoter to the 3'UTR. qPCR analyses estimated 9 copies of the transgene present in the hemizygous and 18 copies in the homozygous rats. In 1-month-old hemizygous P23H-1 rats, transgene expression was 28% less than *Rho* gene in WT rats. ERG showed a progressive rod-cone

dysfunction peaking at 6 months-of-age. SD-OCT confirmed a progressive thinning of the photoreceptor cells layer leading to the disappearance of the outer retina by 6 months, with additional morphological changes in the inner retinal layers in hemizygous P23H-1 rats.
Conclusions: We identified the exact sequence of the transgene inserted in the P23H-1 rat, determined the copy number and evaluated the expression. Functional and structural phenotypic and non-invasive *in vivo* evaluation by SD-OCT and ERG confirms previously reported data.
These results shed light on the genotype of the P23H-1 rat model, better document non-invasively the RP phenotype and provide the foundation for assessing benefit from therapeutic interventions such as gene correction with this model.
Commercial Relationships: Elise Orhan, None; **Deniz Dalkara**, None; **Christophe Lechauve**, None; **Serge A. Picaud**, None; **Thierry D. Leveillard**, None; **Jose A. Sahel**, None; **Muna I. Naash**, None; **Matthew M. LaVail**, None; **Christina Zeitz**, None; **Isabelle S. Audo**, None

Program Number: 5392 **Poster Board Number:** A0241
Presentation Time: 8:30 AM–10:15 AM
Conditional knockout of AMP-activated protein kinase α2 allele leads to retinal cone photoreceptor degeneration
Lei Xu, John D. Ash. Ophthalmology, Univ of Florida, Gainesville, FL.
Purpose: AMP-activated protein kinase (AMPK) acts as an energy sensor to maintain energy homeostasis at the cellular level. It functions as a heterotrimeric protein which consists of one catalytic subunit (α1 or α2), one regulatory subunit (β1 or β2) and one AMP-binding subunits (γ1, γ2, or γ3). Each of these subunits has a specific role in regulating the activity and stability or localization of AMPK. Activation of the AMPK pathway has been demonstrated to be neuroprotective in both light damage and inherited retinal degeneration models. The goal of this study was to determine whether deletion of one of the catalytic subunits AMPK α2 allele in the retina will affect normal retinal function and morphology.
Methods: Chx10 cre: AMPK $\alpha2^{f/f}$ mice are deficient of AMPK α2 in all retinal neurons and Müller glial cells. Retinal function was measured by electroretinography (ERG) and retinal structure was observed by Spectral domain Optical Coherence Tomography (SD-OCT). Western blots and real time PCR were used to study opsin protein and mRNA levels. Immunohistochemistry was used to identify the number of cone photoreceptors. All procedures with animals were conducted in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research.
Results: Conditional knockout of AMPK α2 mice have normal retinal structure, thickness and scotopic ERG a-wave amplitudes. However, they have decreased photopic and flicker ERG responses starting at 2 months, which indicates cone photoreceptor degeneration. Immunohistochemistry using anti-M cone and S cone antibodies indicated a reduction in cone cell numbers at 6 months of age relative to control cre negative mice.
Conclusions: Our data suggest that conditional knockout of AMPK α2 leads to retinal cone photoreceptor degeneration. The data show that AMPK α2 has a unique role in cones, and that the AMPK pathway is essential for cone function and survival. The study further suggests that drugs designed to activate this pathway may have therapeutic benefit for macular degenerative diseases.
Commercial Relationships: Lei Xu, None; **John D. Ash**, None
Support: Funding support to JDA include NIH R01EY016459-10, Foundation Fighting Blindness, and an unrestricted departmental grant from Research to Prevent Blindness, Inc.

Program Number: 5393 **Poster Board Number:** A0242
Presentation Time: 8:30 AM–10:15 AM
Aggressive photoreceptor cell degeneration in PI3KC3/Vps34 conditional knockout mice
Feng He[1], Yan Yin Tse[2], Melina A. Agosto[1], Samuel M. Wu[2], Theodore G. Wensel[1, 2]. [1]Biochemistry, Baylor College of Medicine, Houston, TX; [2]Ophthalmology, Baylor College of Medicine, Houston, TX.
Purpose: The type III phosphoinositide 3-kinase (PI3KC3/Vps34) participates in various cellular functions, including intracellular trafficking and survival. The biological significance of Vps34 in retina is not fully understood. In this study, we investigated Vps34 functions in photoreceptors using rod-specific Vps34 conditional knockout mice.
Methods: A mouse line with a conditional functional deletion of Vps34 in rods was generated. Functional and structural changes in the retina were determined by morphological analysis, western blotting and electroretinography (ERG). An ultrasensitive ELISA-based assay with luminescence detection was used to measure phosphatidylinositol 3-phosphate (PI(3)P) levels. Electroporation of plasmids encoding a PI(3)P binding domain-EGFP fusion was used to localize PI(3)P within rod cells.
Results: PI(3)P localized to discrete puncta of various sizes in the inner segment. Light exposure induced a massive increase of PI(3)P levels in rods, with a slow time course spanning hours. Deletion of Vps34 in rods decreased light-induced PI(3)P levels by more than 94% and caused aggressive retinal degeneration. In contrast, deletion of the type I PI-3 kinase (conditional KO of P85a) had no effect on PI(3)P levels or retinal structure. The number of photoreceptor cells in Vps34 KO mice was decreased by about 50% at 1.5 months and almost none remained at 3 months, while there was no significant change in the inner nuclear or ganglion cell layer. The retinal degeneration was not significantly affected by light. Western blotting of autophagy markers showed dramatic increases in the levels of LC3-II, Rab7, p62, LAMP-1, and LAMP-2 in Vps34 KO mice. Although both LC3 and LAMP-1 accumulated in the inner segments of Vps34 KO mice, they did not co-localize as determined using immunofluorescence microscopy. ERG was normal at 1 month, but exhibited greatly reduced amplitudes of both a- and b-waves at 2 months. Rhodopsin trafficking was examined using Vps34 KO mice which were also heterozygous for a rho-EGFP knock-in. Our results suggest PI(3)P does not significantly contribute to rhodopsin trafficking, as rho-EGFP did not accumulate in the inner segments and ONL in vps34 KO mice.
Conclusions: The type III PI-3 kinase but not the type I kinase, contributes to light-induced synthesis of PI(3)P in rods, and is essential for rod survival. The mechanisms may involve disruption of autophagy and/or related pathways.
Commercial Relationships: Feng He, None; **Yan Yin Tse**, None; **Melina A. Agosto**, None; **Samuel M. Wu**, None; **Theodore G. Wensel**, None
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Program Number: 5394 **Poster Board Number:** A0243
Presentation Time: 8:30 AM–10:15 AM
***TULP1* missense mutations induce the endoplasmic reticulum unfolded protein response (ER-UPR) stress complex.**
Glenn Lobo[1], Adrian Au[1], Lindsey A. Ebke[1], Stephanie A. Hagstrom[1, 2]. [1]Ophthalmic Research, Cleveland Clinic, Cleveland, OH; [2]Ophthalmology, Case Western Reserve University, Cleveland, OH.

Purpose: Mutations in the *TULP1* gene are associated with early-onset retinitis pigmentosa (RP) and Leber congenital amaurosis (LCA). However, the pathologic mechanism causing photoreceptor cell death remains unknown. We hypothesize that *TULP1* mutations produce misfolded protein products that accumulate in the endoplasmic reticulum (ER) and induce cellular apoptosis via the unfolded protein response (UPR) pathway.
Methods: Protein stability of missense *TULP1* mutations were evaluated using the programs PolyPhen, SIFT, I-Mutant and RaptorX. Full-length TULP1 was amplified from human retinal RNA and cloned into the mammalian expression vector pEGFP-N1. The pTULP1-wt-GFP plasmid was used as a template to engineer each of the *TULP1* mutations (R420P, I459K, F491L) by site directed mutagenesis. hTERT-RPE-1 cells were transiently transfected with 3 μg of individual purified DNA plasmids. Six days after transfection, subcellular localization patterns of the GFP-tagged TULP1 proteins were achieved by confocal imaging. Total protein from transfected hTERT-RPE-1 cells was isolated for Western blot analysis.
Results: *In-silico* analyses of known *TULP1* missense mutations predict the mutant proteins will be unstable and misfolded under physiological conditions. Confocal microscopy revealed that GFP-tagged wt TULP1 localized predominantly to the cytoplasm and plasma membrane. In contrast, all three mutant TULP1 proteins showed cytoplasmic punctate staining which co-localized with the ER using ER tracker. Western blot analysis of cells expressing mutant TULP1 proteins revealed an induction of the three key ER stress response proteins, BiP, CHOP and phospho-PERK.
Conclusions: Our *in-silico* and *in-vitro* analyses suggest that mutant TULP1 proteins accumulate within the ER leading to induction of the UPR stress response complex as the pathologic mechanism causing photoreceptor degeneration. However, *in vivo* models are required to further validate this mechanistic pathway and allow for investigation of therapeutics capable of modulating the UPR to aid in the attenuation of *TULP1*-induced photoreceptor cell death.
Commercial Relationships: Glenn Lobo, None; **Adrian Au**, None; **Lindsey A. Ebke**, None; **Stephanie A. Hagstrom**, None

Program Number: 5395 **Poster Board Number:** A0244
Presentation Time: 8:30 AM–10:15 AM
Phenotypic characterization of cats homozygous for a frameshift mutation in *Crx* (*Crx*$^{Rdy/Rdy}$)
Laurence M. Occelli[1], Nicholas M. Tran[3, 4], Kristina Narfstrom[2], Shiming Chen[3], Simon M. Petersen-Jones[1]. [1]Small Animal Clinical Sciences, Michigan State University, East Lansing, MI; [2]Veterinary Medicine and Surgery, University of Missouri-Columbia, Columbia, MO; [3]Ophthalmology and Visual Sciences, Washington University School of Medicine, St. Louis, MO; [4]Molecular and Cellular Biology, Center for Brain Science, Harvard University, Cambridge, MA.
Purpose: CRX is a transcription factor essential for normal photoreceptor development and survival. The *Rdy* cat has a spontaneous frameshift mutation in *Crx*. This type of mutation is similar to Class III *CRX* mutations that result in accumulation of mutant CRX protein which has a dominant negative action. The heterozygous cat (*Crx*$^{Rdy/+}$) has early dysfunction and degeneration of photoreceptors mimicking the severe Leber congenital amaurosis phenotype. This study investigated the phenotype of the homozygous cat (*Crx*$^{Rdy/Rdy}$).
Methods: *Crx*$^{Rdy/Rdy}$ cats were investigated by ophthalmic examination, A-mode ultrasound (US), electroretinography (ERG), optical coherence tomography (OCT) and histology. Molecular changes were assessed by qRT-PCR, Western blot (WB) and immunohistochemistry (IHC).
Results: *Crx*$^{Rdy/Rdy}$ cats lacked vision and showed an absence of menace response and dazzle reflex. They also had a very decreased pupillary light reflex. Unlike the *Crx*$^{Rdy/+}$ cats, the *Crx*$^{Rdy/Rdy}$ cats did not exhibit nystagmus. The globe length was significantly greater than that of wild-type kittens from as early as 1 month of age. Scotopic and photopic ERG responses were absent at all ages tested. *Crx*$^{Rdy/Rdy}$ cats developed tapetal fundus hyperreflectivity detectable as early as 12 weeks of age but there was no obvious thinning of the retinal vasculature. The features of the photoreceptor inner/outer segments (IS/OS) were not discernible on OCT images from an early age. Failure of photoreceptor IS/OS development was confirmed by histology and IHC. Although the other retinal layers appeared relatively normal at 2 weeks of age, retinal stratification became increasingly abnormal with age. QRT-PCR in 2-week-old kittens revealed a decrease in cone and rod opsin mRNA levels while that of *Crx* was elevated. WB revealed that the amount of mutant Crx was greater than that of normal Crx protein in wild-type control retinas.
Conclusions: The retina of the *Crx*$^{Rdy/Rdy}$ cat failed to fully mature resulting in blindness. Also, abnormal globe growth occurs such that the posterior segment of the globe becomes enlarged. Although relatively normal retinal stratification had developed at very early age, lack of normal development of photoreceptor IS/OS was noted and retinal layers became disorganized with disease progression. This was accompanied by a decrease in the expression of rod and cone opsins and absence of retinal function.
Commercial Relationships: Laurence M. Occelli, None; **Nicholas M. Tran**, None; **Kristina Narfstrom**, None; **Shiming Chen**, None; **Simon M. Petersen-Jones**, None
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Program Number: 5396 **Poster Board Number:** A0245
Presentation Time: 8:30 AM–10:15 AM
Pattern of retinal morphological and functional decay in Tvrm4 rhodopsin mutant mice
Enrica Strettoi[1], Elena Novelli[1], Ilaria Piano[2], Claudia M. Gargini[2]. [1]CNR Neuroscience Institute, Pisa, Italy; [2]Department of Pharmacy, University of Pisa, Pisa, Italy.
Purpose: Genetic variability is a hallmark of Retinitis Pigmentosa (RP), with known mutations in over 60 different genes. Rhodopsin (RHO) mutations prevail and cause 25%- 40% of the dominant cases. Here we describe the phenotype of Tvrm4 mice, which carry a dominant RHO mutation activated upon brief exposure to strong, white light. Tvrm4 mice, devised in the laboratory of Dr. P. Nishina at Jackson's (Budzynski et al., 2010), make it possible to trigger phenotype manifestation outside the period of retinal development, in young adulthood, mimicking the age of onset of typical human RP.
Methods: Tvrm4 mice (with a I307N mutation of RHO) aged 2-4 months and wt littermates were given eye drops of atropine, placed in an illuminating box built *ad hoc* and exposed to pulses (1', 2' or 3') of 12,000 Lux white neon light. After 48 hrs, 7, 14 or 21 days, mice were used for conventional recordings of flash scotopic and photopic ERG. Additional mice were harvested at 48 hrs, 7 and 14 days, their eyes enucleated, fixed and processed for immunocytochemistry and confocal microscopy. Antibodies were used on retinal whole mounts and frozen sections to label rods, cones, bipolar and glial cells.
Results: Exposure of Tvrm4 mice to 12,000 Lux light for either 1', 2' or 3' results in a typical rod-cone degeneration propagating with a center to periphery retinal gradient. Rod outer segments shorten after 48 hrs and their fragments accumulate in the subretinal space. The ONL becomes thinner. Cones degenerate more slowly, first shortening and loosing spatial regularity. Micro and microglial activation and

dendritic retraction in the OPL accompany photoreceptor loss. ERG regression is visible at all ages tested, with decrement of scotopic responses preceding that of photopic signals. Upon 2' exposure, after 14 days, scotopic a waves are undetectable, reduced b wave persist and a concomitant decrement of oscillatory potentials is observed. Severity of phenotype increases in time and appears directly related to the duration of bright light exposure.
Conclusions: Unlike other RHO models of RP, Tvrm4 mice are not transgenics; they are inducible, show a typical rod-cone degeneration and a sensitivity to light common to various forms of retinal degeneration. Hence, these mice are an excellent model of RP and can be used to study mechanisms of cell death associated to dominant mutations of RHO as well as to test rescue strategies for human RP.
Commercial Relationships: Enrica Strettoi, None; **Elena Novelli**, None; **Ilaria Piano**, None; **Claudia M. Gargini**, None
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Program Number: 5397 **Poster Board Number:** A0246
Presentation Time: 8:30 AM–10:15 AM
An Activated Unfolded Protein Response Triggers Retinal Degeneration in the Wild Type Mice Via Activation of Inflammatory Response.
Marina S. Gorbatyuk[1], Tapasi Rana[1], Vishal M. Shinde[1], Christopher Starr[1], Evan Boitet[1], Pravalika Kotla[1], Sergei Zolotukhin[2], Alecia K. Gross[1]. [1]Vision Sciences, University of Alabama at Birmingham, Birmingham, AL; [2]Pediatrics, University of Florida, Gainesville, FL.
Purpose: Recently it has been shown that the UPR is involved in the pathogenesis of many retinal degenerative diseases. However, the main question of whether UPR activation actually triggers retinal degeneration remains to be addressed. The goal of this study is to verify the role of persistently activated UPR in retinal pathogenesis.
Methods: C57BL6, T1117M Rho (ClassI) and Ter349Glu (ClassII) mice were used in the study. A mouse model of retinal degeneration caused by an activated UPR was created by injecting the C57BL6 mice with tunicamycin. ERG, SD-OCT and histological analysis were used to assess the physiological and morphological parameters associated with this disease. A potential mechanism by which the UPR triggers the retinal degeneration was delineated using the retinal RNA and protein extracts.
Results: We detected a significant loss of wild type photoreceptor function (over 60%) and retinal structure (35%) 30 days post treatment. Analysis of retinal protein extracts demonstrated significant up-regulation of inflammatory markers IL-1β, IL-6, TNFα, MCP-1 and IBA1. Similar up-regulation of pro- and anti-inflammatory markers was found in the T17M RHO mice experiencing the UPR activation. The UPR activation was also found in other model of severe retinopathy expressing the Ter349Glu Rhodopsin though a protein misfolding is not a primary cause for UPR activation in this model. Interestingly, both mouse models demonstrated the activation of F4/80 and IBA1 microglial markers suggesting a link between the UPR activation and the inflammatory signaling. We then verified this link by testing a hypothesis of whether the early UPR-mediated IL-1β could be responsible for promotion of retinal degeneration. Approximately 19% reduction in the scotopic ERG a-wave amplitudes and a 29% loss of photoreceptor cells compared to control retinas were observed in the C57BL6 mice subretinaly injected with recombinant IL-1b, suggesting a potential link between pro-inflammatory cytokines and retinal pathophysiological effects.
Conclusions: Our work demonstrates that in the context of an established animal model for ocular disease, the persistent activation of the UPR could be responsible for promoting retinal degeneration via the UPR-induced pro-inflammatory response.
Commercial Relationships: Marina S. Gorbatyuk, None; **Tapasi Rana**, None; **Vishal M. Shinde**, None; **Christopher Starr**, None; **Evan Boitet**, None; **Pravalika Kotla**, None; **Sergei Zolotukhin**, None; **Alecia K. Gross**, None
Support: RO1EY020905; R01EY019311

Program Number: 5398 **Poster Board Number:** A0247
Presentation Time: 8:30 AM–10:15 AM
Retinal degeneration caused by deficient mitochondrial transcription factor A in murine photoreceptors
kiyohito Totsuka, Murilo F. Roggia, Takashi Ueta. ophthalmology, The university of Tokyo, Tokyo, Japan.
Purpose: Patients with mitochondrial diseases sometimes presented with retinitis pigmentosa, while the defects in mitochondrial DNA or functions specifically in photoreceptors has been unclear. Herein, to investigate the effect of mitochondrial defects in murine photoreceptors *in vivo*, we generated photoreceptor-specific conditional knockout (CKO) mice of mitochondrial transcription factor A (TFAM).
Methods: We generated CKO mice of TFAM by crossing $TFAM^{flox/flox}$ mice and Crx-Cre mice, while used $TFAM^{flox/flox}$ littermate mice as control. We examined their retina by immunohistochemical analyses at postnatal day 3 (P3), day 7 (P7), day 13 (P13), and 2 months (2M). Retinal morphology was assessed by Hematoxylin-Eosin staning. Deficient TFAM expression in photoreceptors of CKO mice was examined by immunohistochemistry for TFAM protein. The development and survival of photoreceptors in CKO mice were investigated by immunohistochemistry for retinoic acid receptor-γ (Rxr-γ), rhodopsin, m-opsin and s-opsin. TUNEL assay and immunostaining for cleaved caspase 3 and apoptosis inducing factor (AIF) were used for the evaluation of photoreceptor death in CKO mice.
Results: As we expected, TFAM protein expression was adequately abrogated in only photoreceptors in retinas of CKO mice. We confirmed that the retinas in CKO mice generated the outer nuclear layer (ONL), although thinner than the ONL of the control mice at P7. After P7 photoreceptors of CKO mice degenerated with TUNEL-positive cell death. The apoptosis in photoreceptors were observed with the cleaved caspase 3, but not with the nuclear translocation of AIF, suggesting capase-dependent apoptosis. In regard to photoreceptor differentiation, Rxr-γ-labeled cells were similarly observed in outer neuroblastic layer of both CKO and control mice at P3. At P13 and 2M, while rhodopsin was expressed in the outer segment of photoreceptors, m- and s- opsin protein expression was significantly diminished.
Conclusions: Photoreceptor-specific CKO mice of TFAM presented with retinal degeneration, specifically cone photoreceptor degeneration. The CKO mice could be an *in vivo* model for the photoreceptor pathologies related to mitochondrial dysfunctions.
Commercial Relationships: kiyohito Totsuka, None; **Murilo F. Roggia**, None; **Takashi Ueta**, None

Program Number: 5399 **Poster Board Number:** A0248
Presentation Time: 8:30 AM–10:15 AM
***In vivo* correlation of retinal morphological and functional changes in a porcine model of inherited retinal degeneration**
Wankun Xie[1], Gavin W. Roddy[2], Patrick D. Bradley[3], Shu-Huai Tsai[1], Luke B. Potts[3], Wenjuan Xu[1], Christina Du[4], Travis W. Hein[1, 3], Lih Kuo[1, 3], Robert H. Rosa[1, 3]. [1]Surgery and Medical Physiology, Texas A&M Health Science Center, Temple, TX; [2]Ophthalmology, Mayo Clinic, Rochester, MN; [3]Ophthalmology, Scott & White Eye Institute, Temple, TX; [4]Comparative Medicine, Baylor Scott & White Health, Temple, TX.
Purpose: Retinitis pigmentosa (RP) is a group of inherited diseases that cause severe visual dysfunction due to the progressive degeneration of rod and cone photoreceptors. Inherited mutations in the rhodopsin gene are the most common causes of autosomal dominant RP, including the Pro23His substitution leading to protein misfolding. Although a Pro23His rhodopsin transgenic miniature pig model has recently been developed, the morphological correlation with functional changes remains limited. Herein, we addressed this issue using spectral domain optical coherence tomography (SD-OCT) and electroretinography (ERG) in this experimental model.
Methods: Pro23His rhodopsin transgenic and wild-type hybrid pig littermates were obtained from the National Swine Resource and Research Center, University of Missouri-Columbia. Retinal morphology was assessed *in vivo* by SD-OCT between postnatal days (P) 30 and 120. Retinal function was evaluated using full-field ERG simultaneously (P30 to P120) in the same animal subjects. Morphological and ERG data were correlated with time in this porcine model of retinal degeneration.
Results: Retinal outer nuclear layer (ONL) thickness as measured by SD-OCT was significantly reduced (75%, $p<0.05$) at P60 in the transgenic pig compared to wild-type. A further reduction in ONL thickness occurred at P75 (86.6%, $p<0.05$), without significant change in ONL thickness between P75 and P120. The mean scotopic ERG a-wave and b-wave amplitudes (at a light stimulus of 3.0 $cdsm^{-2}$) decreased by 84.4% ($p<0.05$) and 77.6% ($p<0.05$), respectively between P30 and P90, with amplitudes remaining relatively stable between P90 and P120. The mean photopic ERG a-wave and b-wave amplitudes (at a light stimulus of 3.0 $cdsm^{-2}$) decreased by 62.4% and 60.4%, respectively between P30 and P90, with amplitudes remaining relatively stable between P90 and P120.
Conclusions: In this study, we correlated the *in vivo* morphological and functional changes over time (P30-P120) in a recently described porcine model of retinal degeneration. Our results are consistent with the previous findings, with the addition of novel *in vivo* data utilizing SD-OCT. These data provide a foundation for future studies investigating potential new therapeutic strategies to rescue photoreceptor degeneration.
Commercial Relationships: Wankun Xie, None; **Gavin W. Roddy**, None; **Patrick D. Bradley**, None; **Shu-Huai Tsai**, None; **Luke B. Potts**, None; **Wenjuan Xu**, None; **Christina Du**, None; **Travis W. Hein**, None; **Lih Kuo**, None; **Robert H. Rosa**, Genentech (F)
Support: Scott & White Healthcare Foundation

Program Number: 5400 **Poster Board Number:** A0249
Presentation Time: 8:30 AM–10:15 AM
Degenerative phenotype observed in retinal pigment epithelium of *Prpf31*$^{A216P/+}$ mice is caused by an abnormal pre-mRNA splicing of *Rpe65* gene
Francisco J. Diaz-Corrales, Berta De la Cerda, Lourdes Valdes-Sanchez, Daniel Rodriguez-Martinez, Ana Aramburu, Eduardo Rodriguez-Bocanegra, Ana Belén García-Delgado, Shom S. Bhattacharya. Cell Therapy, CABIMER, Seville, Spain.
Purpose: Mutations in ubiquitously expressed pre-mRNA splicing-factor genes *PRPF3*, *PRPF8*, and *PRPF31* cause autosomal dominant retinitis pigmentosa (adRP). It has been reported that transgenic mouse models carrying mutations in these genes exhibit morphological changes in the retinal pigment epithelium (RPE) (Graziotto et al. IOVS 2011). The reasons why these genes produce a degenerative phenotype in the RPE are unknown. Thus, we deeply characterized a heterozygous knock-in (KI) mouse model carrying the mutation A216P in the *Prpf31* gene (*Prpf31* $^{A216P/+}$) to better understand the molecular mechanism involved in this form of adRP.
Methods: Eight-month-old C57BL/6J *Prpf31* $^{A216P/+}$ KI mice and wild type (WT) littermates were used in all experiments. Spatial vision (visual acuity and contrast sensitivity) was evaluated by optomotor test (OT) using 6 spatial frequencies from 0.031 to 0.272 cycles/degree (c/d) at 100%, 75% and 50% contrast sensitivity. The c-wave amplitude, which is originated in the RPE, was measured in a Ganzfeld electroretinogram (ERG). Retinal thickness and RPE reflectivity were quantified by optical coherence tomography (OCT). Western blotting (WB), RT-PCR, and cDNA sequencing were used to detect mRNA splicing errors in visual cycle genes. Fundus and histology of RPE were performed to detect lipofuscin accumulation.
Results: 1) OT: the percentage of positive responses at 100% to 50% contrast sensitivity in all spatial frequencies tested is diminished in the KI mice (100% contrast sensitivity, spatial frequency 0.272 c/d, WT= 70 ± 3 %, KI= 38 ± 2 %, $p< 0.01$). 2) ERG: the mean of c-wave amplitude is smaller in KI mice (WT= 283 ± 7 μV, KI= 190 ± 25 μV, $p< 0.05$). 3) OCT: the mean KI retina is thinner (WT= 220 ± 5 μm, KI= 198 ± 5 μm $p< 0.05$). 4) WB, RT-PCR and cDNA sequencing demonstrate an abnormal pre-mRNA splicing in exons 3 and 9 of Rpe65 gene. 5) Fundus evaluation and histology of RPE show an increase of autofluorescence and accumulation of lipofuscin granules.
Conclusions: *Prpf31*$^{A216P/+}$ mice have been previously characterized, without a clear phenotype of photoreceptor degeneration (Bujakowska et al. IOVS 2009). However, we have found that these mice have a visual dysfunction and degenerative phenotype in the RPE which is caused by an abnormal pre-mRNA splicing of Rpe65 gene.
Commercial Relationships: Francisco J. Diaz-Corrales, None; **Berta De la Cerda**, None; **Lourdes Valdes-Sanchez**, None; **Daniel Rodriguez-Martinez**, None; **Ana Aramburu**, None; **Eduardo Rodriguez-Bocanegra**, None; **Ana Belén García-Delgado**, None; **Shom S. Bhattacharya**, None
Support: CSIC-Andalusian Government P09-CTS-4697 and ISCIII-FPS113/12

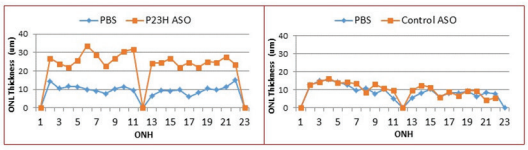
Program Number: 5401 **Poster Board Number:** A0250
Presentation Time: 8:30 AM–10:15 AM
Deficiency of Intraflagellar Transport Protein 139 in Rod Photoreceptors Causes Early-Onset Rod-Cone Degeneration in Mice
Scott H. Greenwald, Magdalena M. Staniszewska, Eric A. Pierce, Qin Liu. Ophthalmology, Massachusetts Eye and Ear Infirmary, Boston, MA.
Purpose: The specific role of retrograde intraflagellar transport protein 139 (IFT139), encoded by the *TTC21B* gene, in the development and viability of ciliated cells has yet to be defined. However, mutations in *TTC21B* have been linked to cilopathies with retinal phenotypes such as nephronophthisis and Jeune asphyxiating thoracic dystrophy. To investigate how this protein influences ciliated cell survival, including that of photoreceptors, a rod-specific conditional knockout mouse line was generated. Changes in retinal morphology and function were assessed in these mice, *in vivo*.

Methods: Mice engineered to have a targeted *Ttc21b* allele with loxP sites flanking exon 4 were crossed with rhodopsin-iCre transgenetic mice to knock out IFT139 in rods. All experiments performed on the *Ttc21b*$^{flox/flox}$/*Rho-iCre*$^{+}$ conditional knockout mice (cKO) were compared to litter-mate *Ttc21b*$^{flox/flox}$ control mice. Retinal function was measured by full-field electroretinography (ERG) using a broadband 4ms ON-OFF stimulus in dark-adapted and light-adapted conditions. Retinal morphology was assessed using fundus photography and optical coherence tomography (OCT).
Results: The cKO mice exhibited an early-onset retinal degeneration that resulted in a dramatic loss of photoreceptors by two months of age. At one month, the fundi of the cKO mice appeared normal, although OCT revealed that retinas were 22% ($p=7x10^{-13}$) thinner than those of control mice due to deterioration of the photoreceptor layer. At this age, ERG demonstrated that rod function was 55% ($p<0.001$) lower in cKO mice, whereas cone function had just started to decrease (-8%, $p=0.37$). At two months, fundus imaging in the cKO mice showed attenuated vasculature, abnormal optic disc pallor, and RPE that was visible through the neural retina; the cKO retinas were 43% ($p=2x10^{-12}$) thinner than those of control mice. Neither rod nor cone activating stimuli generated a detectable ERG in the two month old cKO mouse.
Conclusions: Rod photoreceptors with a deficiency of IFT139 developed and were still moderately functional in one month old mice. However, by two months, rod degeneration was widespread and, in this environment, the cones also failed. Future experiments will investigate, at the mechanism level, how IFT139 contributes to ciliated cell survival.
Commercial Relationships: Scott H. Greenwald, None; **Magdalena M. Staniszewska**, None; **Eric A. Pierce**, None; **Qin Liu**, None
Support: NEI Grant EY012910, Foundation Fighting Blindness, Research to Prevent Blindness

Program Number: 5402 **Poster Board Number:** A0251
Presentation Time: 8:30 AM–10:15 AM
Identification of a Human Clinical Candidate ASO Selectively Targeting the P23H Variant of Rhodopsin for the Treatment of P23H Rhodopsin-mediated ADRP
Sue F. Murray[1], Ali Jazayeri[1], Raechel Peralta[1], Andy Watt[1], Timothy Vickers[1], Shuling Guo[1], Scott P. Henry[1], Peter S. Adamson[2], Brett P. Monia[1], Michael McCaleb[1]. [1]Exploratory Research, ISIS Pharmaceuticals, Carlsbad, CA; [2]Ophthiris, GlaxoSmithKline, Stevenage, United Kingdom.
Purpose: To identify an allele-specific antisense oligonucleotide (ASO) targeting the P23H variant of human rhodopsin mRNA (hRHO), the most common cause of ADRP in American patients. P23H RHO ASO will be selective for the P23H allele, sparing normal (WT) mRNA expression, resulting in a reduction of the mutant protein and allowing WT protein expression and processing, and maintenance of rod survival and function.
Methods: Cell lines expressing human WT and P23H RHO sequences were used to identify potent and selective P23H RHO ASOs. Leads were evaluated in vivo using transgenic (Tg) mice expressing one allele of the P23H hRHO gene or Tg mice expressing one allele of the human WT RHO gene after administration of either a human P23H specific ASO or a control non-rhodopsin ASO by intravitreal injection (IVT). To assess therapeutic potential of the ASO, the lead P23H RHO ASO was evaluated for activity and selectivity in cynomolgus monkeys.
Results: ISIS 664844 had an in vitro IC_{50} of 2uM in P23H hRHO expressing cells and >40uM in cells expressing WT hRHO. The ASO targeting the human P23H RHO sequence achieved a 42±5% reduction in mutant RHO mRNA expression 7d following a 50 μg IVT injection in the P23H RHO Tg mice. There was no reduction in the control non-rhodopsin ASO treated eyes (0±3%). ISIS 664844, did not significantly reduce human WT RHO expression. Evaluation of 664844 in monkeys demonstrated no significant reduction in monkey WT RHO expression at 150 μg (3 ± 3%; $p>0.05$) 10 wk after a single IVT injection as compared to PBS-injected eyes. As a positive control, a monkey active ASO targeted against the WT RHO sequence achieved a 60±7% reduction after a single IVT injection at 400 μg. No structural or functional changes were observed in the eyes treated with 664844, as determined by ophthalmological exam, histological exam or ERG analysis.
Conclusions: We have identified and characterized a human RHO P23H allele-specific ASO, ISIS 664844, which targets the P23H RHO allele without significantly affecting the expression of the WT RHO allele. The P23H mutation causes a nonfunctional protein with a toxic gain of function and a dominant negative effect on normal protein. Therefore, reduction of the mutant protein, while sparing the RHO WT protein, should diminish the rate of degeneration associated with adRP and preserve vision for a longer period of time.
Commercial Relationships: Sue F. Murray, Isis Pharmaceuticals (E); **Ali Jazayeri**, Isis Pharmaceuticals (E); **Raechel Peralta**, Isis Pharmaceuticals (E); **Andy Watt**, Isis Pharmaceuticals (E); **Timothy Vickers**, Isis Pharmaceuticals (E); **Shuling Guo**, Isis Pharmaceuticals (E); **Scott P. Henry**, Isis Pharmaceuticals (E); **Peter S. Adamson**, GlaxoSmithKline (E); **Brett P. Monia**, Isis Pharmaceuticals (E); **Michael McCaleb**, Isis Pharmaceuticals (E)

Program Number: 5403 **Poster Board Number:** A0252
Presentation Time: 8:30 AM–10:15 AM
Amelioration of Photoreceptor Degeneration in a Mouse Model of P23H ADRP Using an Antisense Oligonucleotide (ASO)
Ali Jazayeri, Sue F. Murray, Raechel Peralta, Audrey Low, Bea DeBrosse-Serra, Shuling Guo, Brett P. Monia, Michael McCaleb. Antisense Discovery, ISIS, Carlsbad, CA.
Purpose: Antisense technology provides an ideal platform for the treatment of autosomal dominant genetic diseases in the eye. We explored the pharmacological benefits of ASO mediated reduction of human P23H transgene mRNA in a mouse model of P23H ADRP.
Methods: Transgenic (Tg) mice harboring human rhodopsin genomic sequence with the P23H variant were used to evaluate efficacy (mRNA knockdown) and pharmacology (ONL thickness) of ASOs by IVT delivery. Tg mice were dosed intravitreally with 1 uL of either PBS, 50 ug of P23H ASO or control ASO and sacrificed 7-60 d later. The eyes were fixed for ONL thickness analysis from histological sections or total RNA prepared for RT-PCR analysis.
Results: **RESULTS:** A single IVT dose of P23H ASO at 50 ug resulted in 57±8% inhibition of the P23H rho mRNA at 30 d while the control ASO had no effect. In P23H homozygote mice (various ages), this single dose of P23H ASO resulted in 57±7% preservation of the ONL layer when compared to the contralateral eye (CLE) at 60 d after treatment. Similar effects were seen in all age groups studied (1-3 months of age at the time of treatment). Eyes injected with P23H ASO had significantly more mouse rhodopsin mRNA (200 ± 18%) vs. CLE and a 47±7% increase in mouse to human rhodopsin ratio as compared to control ASO which are consistent with the presence of larger numbers of photoreceptor cells.
Conclusions: Altering the ratio of mutant to wild type rhodopsin is an effective method of photoreceptor cell preservation and treatment for P23H ADRP. This is highlighted by age and degenerative state independent increases in ONL survival observed in these studies.

Further, long duration of action of ASOs (>60 days) provide a potential added benefit of less frequent dosing in patients.



Commercial Relationships: Ali Jazayeri, isis pharmaceuticals (E); **Sue F. Murray**, ISIS Pharmaceuticals (E); **Raechel Peralta**, isis pharmaceuticals (E); **Audrey Low**, ISIS Pharmaceuticals (E); **Bea DeBrosse-Serra**, ISIS Pharmaceuticals (E); **Shuling Guo**, ISIS Pharmaceuticals (E); **Brett P. Monia**, ISIS Pharmaceuticals (E); **Michael McCaleb**, ISIS Pharmaceuticals (E)

Program Number: 5404 **Poster Board Number:** A0253
Presentation Time: 8:30 AM–10:15 AM
TNFα-deficiency mitigates the retinal degeneration in T17MRHO
Tapasi Rana, Marina S. Gorbatyuk. Vision Sciences, University of Alabama, Birmingham, AL.
Purpose: Previous studies have shown that several inflammatory events such as the TNFα, NFkB, IL-1β, IL-6 and MCP-1 elevation, accompanied by microglia activation (F4/80 and Iba1), are involved in the photoreceptor degeneration of T17M rhodopsin (*RHO*) mice mimicking the human autosomal dominant retinitis pigmentosa (ADRP). These results have suggested that the TNF-α pathway plays an important role in the pathogenesis of ADRP retinas and the impact of TNFα in the ADRP ocular pathology has to be verified. Therefore, we investigated the role of TNF-α and TNFa-activated cellular signaling in the retinal pathogenesis of T17M*RHO* mice.
Methods: T17M*RHO*, $TNF^{+/-}$, T17M*RHO* $TNF^{+/-}$ and C57BL6 mice were involved in the study. ERG and OCT were performed for all groups at P30, P60 and P90.
Results: Analysis of the scotopic ERG recording demonstrated that the a-wave amplitudes in the T17M RHO $TNF\alpha^{+/-}$ mice were significantly increased by 429%, 229%, 217% at P30, P60 and P90, respectively when compared to T17MRHO littermates. At P30, no difference between the T17M RHO TNFα and the C57BL6 animals were recorded. Interestingly, that the b-wave ERG amplitudes were more prominently preserved in the T17M RHO TNFα. Significant elevation of the b-wave amplitudes by 234%, 157% and 131%, respectively at P30, P60 and P90 were detected in the T17MRHO $TNF\alpha^{+/-}$ animals that was compatible with the amplitudes registered in the wild type retinas at P30 and P60. The SD-OCT analysis supported the ERG data and demonstrated that the average ONL thickness of the superior and inferior retinas measured within 400 nm from the optic nerve head (ONH) was significantly by 151% enhanced in the T17M*RHO* $TNF\alpha^{+/-}$ mice at P30 as compared to ADRP control.
Conclusions: This study demonstrates that the TNFα-deficiency mitigates the retinal degeneration in T17M RHO mice and that the TNFα may be a promising therapeutic target to prevent loss of ADRP photoreceptor function and cell death.
Commercial Relationships: Tapasi Rana, None; **Marina S. Gorbatyuk**, None
Support: RO1EY020905

Program Number: 5405 **Poster Board Number:** A0254
Presentation Time: 8:30 AM–10:15 AM
ERG preservation by intravitreal injected recombinant mesencephalic astrocyte-derived neurotrophic factor(MANF) in *rd*10 mice
Yiwen Li, Rong Wen. Bascom Palmer Eye Institute, University of Miami, Miami, FL.
Purpose: Mesencephalic astrocyte-derived neurotrophic factor (MANF) is a neurotrophic factor first identified from the conditional medium of a rat type-1 astrocyte cell line, the ventral mesencephalic cell line 1 (VMCL1). We have shown previously that recombinant human MANF protects photoreceptors in a retinal degeneration rat model carrying the S334ter rhodopsin mutation. In the present work, we examined the effect of MANF on the electrophysiolocal activities of retinal cells in the *rd10* mice, a retinal degeneration model carrying the $Pde6b^{rd10}$ mutation.
Methods: The *rd10* mice were kept at ~50 lx 12:12 light:dark cycle. Recombinant human MANF was expressed in E. coli and purified. At PD18 (postnatal day 18), MANF (2 μg in 1 μl) was intravitreally injected to the right eyes of *rd10* mice, and the left eyes were injected with PBS (phosphate buffered saline) as controls. Scotopic ERG responses were recorded 10 days later using an LKC UTAS system. Dark-adapted animals were anesthetized with a mixture of ketamin and xylazine. ERGs were elicited by white light flashes of -0.4 log cd-s/m^2. The b-wave amplitudes of the treated eyes were compared with those of the control eyes. Statistical significance between the two groups was determined by Student *t* test.
Results: The a-wave was undetectable at -0.4 log cd-s/m^2 in the *rd10* mice at PD28. In the control eye injected with PBS, the b-wave amplitude was 82.4±10.7 μV (mean±SD, n=5). The b-wave in the treated eye was 115±16.5 μV (n=5, *P*=0.0017), significantly higher than the control.
Conclusions: Intravitreal injection of recombinant human MANF significantly preserves b-wave in the *rd10* mice. Our results thus demonstrate that MANF not only preserves photoreceptor cells, but also their light-sensing function.
Commercial Relationships: Yiwen Li, None; **Rong Wen**, None
Support: Supported by NIH grants R01EY023666, P30-EY014801, Adrienne Arsht Hope for Vision fund, and an unrestricted grant from Research to Prevent Blindness, Inc.

Program Number: 5406 **Poster Board Number:** A0255
Presentation Time: 8:30 AM–10:15 AM
Repeated synchronous photoactivation of mutant rhodopsin molecules induces rapid retinal degeneration in a T4K rhodopsin model of RP.
Beatrice M. Tam, Orson L. Moritz, Serena Chan. Ophthalmology and Visual Sciences, University of British Columbia, Vancouver, BC, Canada.
Purpose: The rhodopsin mutation T4K is associated with sector RP in humans and causes light dependent retinal degeneration in transgenic X. laevis. Previously we demonstrated that this retinal degeneration is associated with photoactivation of the mutant protein.

Our objective is to elucidate the pathogenic mechanisms underlying the light dependence of this RP model.
Methods: Transgenic lines of X. laevis were mated to produce tadpoles expressing human T4K rhodopsin in their rod photoreceptors. Transgenic tadpoles and their nontransgenic siblings were raised for 14 days in constant dark (which protects the retina from degeneration) and then exposed to varying light regimens in which we altered the light cycle frequency and/or light intensity. Following light exposure, animals were killed and one eye was processed for histology and the other for protein analysis.
Results: We compared retinal degeneration in T4K rhodopsin transgenic X. laevis under conditions of bright constant light, constant dark, and bright cyclic light, as well as constant intermediate and dim light intensities. We found that the retinal degeneration was significantly more severe in cyclic light than any other condition, and both constant dark and constant light were protective relative to cyclic light. We further varied the cyclic light interval, and found that degeneration was maximal in one hour on/one hour off conditions. Interestingly, more frequent intervals were less damaging suggesting that multiple rounds of rhodopsin regeneration and activation are required for cellular toxicity. In animals exposed to 1:1 cyclic light. Retinal degeneration was largely complete within 48 hours. Constant dim light was more protective than constant bright light.
Conclusions: We have identified an unusual retinal degeneration that is protected by both dark rearing and rearing in constant light. Our results suggest that retinal degeneration requires repeated simultaneous activation of a substantial proportion of the mutant rhodopsin present in photoreceptor outer segments.
Commercial Relationships: Beatrice M. Tam, None; **Orson L. Moritz**, None; **Serena Chan**, None
Support: CIHR

Program Number: 5407 **Poster Board Number:** A0256
Presentation Time: 8:30 AM–10:15 AM
Opposing effects of HDAC inhibitors in hP23H and hT17M animal models of retinitis pigmentosa
Ruanne Y. Lai, Zusheng Zong, Beatrice M. Tam, Christopher G. May, Orson L. Moritz. Ophthalmology & Visual Sciences, Vancouver General Hospital, UBC, Vancouver, BC, Canada.
Purpose: Retinitis pigmentosa (RP) is an inherited retinal degenerative disorder for which no treatment is available. Mutations in the rhodopsin gene have been linked to RP. Valproic acid (VPA) is a proposed RP treatment, with two phase II clinical trials underway. However, the mechanism of VPA for RP, if any, is unclear. Therefore, we investigated the effect of VPA on transgenic *X. laevis* models of RP expressing human P23H (hP23H) and hT17M rhodopsins. To elucidate origin of the effects of VPA, we tested structural and functional analogs of VPA, including mood stabilizers, molecular chaperones and HDAC inhibitors.
Methods: Transgenic and wildtype *X. laevis* tadpoles were raised in cyclic light or complete darkness and treated with VPA or other analogs of VPA. Treatment began on post-fertilization day 2 and continued through day 14, after which the animals were sacrificed and genotyped. One eye was solubilized and rhodopsin level was analyzed. The contralateral eye was fixed, cryosectioned, and imaged by confocal microscopy.
Results: We found that VPA and the HDAC inhibitor, sodium butyrate (NaBu), independently rescued RD associated with hP23H rhodopsin dose-dependently. Effect of VPA was mediated by clearing of the mutant hP23H rhodopsin from rod inner segments. The magnitude of the rescue effect from both compounds was equal to that of dark-rearing, but the effect of combined drug treatment and dark-rearing was not additive. Contrastingly, VPA and NaBu dramatically exacerbated RD in hT17M rhodopsin animals in cyclic light conditions, but this detrimental effect was not observed in dark-reared animals. Effects of other structural and functional analogs of VPA were not significant.
Conclusions: VPA and NaBu treatments both rescue RD caused by hP23H rhodopsin, but exacerbate RD caused by hT17M rhodopsin. VPA or NaBu treatment combined with light restriction were not synergistic in hP23H animals, while VPA or NaBu exacerbation of RD caused by hT17M rhodopsin required light exposure. VPA treatment decreases the burden of misfolded hP23H rhodopsin in rod photoreceptors. Both compounds are HDAC inhibitors, and this activity appears to be responsible for both the beneficial and detrimental effects. Our results indicate that the success or failure of pharmacological therapy in RP is likely to be highly dependent on the underlying genotype, and that VPA treatment will be contraindicated for some RP cases.
Commercial Relationships: Ruanne Y. Lai, None; **Zusheng Zong**, None; **Beatrice M. Tam**, None; **Christopher G. May**, None; **Orson L. Moritz**, None
Support: CIHR

Program Number: 5408 **Poster Board Number:** A0257
Presentation Time: 8:30 AM–10:15 AM
Evaluation of the efficacy of mouse opsin promoter for rhodopsin gene augmentation in dogs
Raghavi Sudharsan[1], Simone Iwabe[1], Tatyana Appelbaum[1], Alfred S. Lewin[2], William W. Hauswirth[3], Gustavo D. Aguirre[1], William A. Beltran[1]. [1]Clinical Studies, University of Pennsylvania, Philadelphia, PA; [2]Molecular Genetics and Microbiology, University of Florida, Gainesville, FL; [3]Ophthalmology, University of Florida, Gainesville, FL.
Purpose: An AAV2/5 carrying human Rhodopsin (*hRHO*) cDNA under the control of a mouse opsin promoter (mOP) did not prevent light-induced retinal degeneration in the light-sensitive canine model of autosomal dominant Retinitis Pigmentosa (ADRP) (Iwabe et al, ARVO 2014). We now have evaluated in canine retinas the levels of rhodopsin expression following treatment with this same viral vector construct to determine the lack of rescue effect.
Methods: Wild-type dogs were used; an AAV2/5-mOP-*hRHO* construct was subretinally injected (150 ul at $5x10^{12}$ vg/ml) in 5 eyes, and one eye received equal volume of BSS. Dogs were euthanatized 8-9 weeks post injection. Punches of neuroretina (3 mm diameter) were collected from the bleb (treated) and non-injected areas, and used to analyze levels of expression of *hRHO* transgene by western blot and qRT-PCR. RHO protein levels were evaluated using an antibody that recognizes both canine and human proteins. Transcription of *hRHO* was evaluated by RT-PCR using a set of primers specific to *hRHO*. In addition, absolute quantitation was performed using the qRT-PCR Standard Curve method to determine the absolute amounts of human versus endogenous canine *RHO* mRNA transcribed.
Results: No significant differences in total RHO protein amounts between treated and non-treated regions were found by western blotting. Transcription of *hRHO* in bleb regions retina was confirmed based on Ct values obtained with *hRHO* specific primers. However, using absolute quantitation, we determined that the *hRHO* transgene was being transcribed at only 1-4% of the mRNA levels of the endogenous canine *RHO*.
Conclusions: A similar AAV construct as used here has been previously shown to efficiently augment RHO expression in the transgenic P23H mouse model of ADRP, yielding a two-fold increase in total *RHO* mRNA, and a 1.5 times increase in protein (Mao et. al., Hum. Gene Ther., 2011). In the canine retina, our current results

show that *hRHO* transgene expression is low, thus explaining the lack of rescue effect in the canine T4R *RHO* model of ADRP that we had previously reported. This may be due either to limited efficiency of the mouse opsin promoter, or to instability of *hRHO* transcripts in the canine retina. Ongoing studies now focus on the human opsin promoter instead, and adding enhancer elements to the viral construct to improve transgene expression.
Commercial Relationships: Raghavi Sudharsan, None; **Simone Iwabe**, None; **Tatyana Appelbaum**, None; **Alfred S. Lewin**, None; **William W. Hauswirth**, AGTC Inc. (P); **Gustavo D. Aguirre**, None; **William A. Beltran**, None
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Program Number: 5409 **Poster Board Number:** A0258
Presentation Time: 8:30 AM–10:15 AM
Controlling PERK-ATF4-CHOP branch of the UPR is the key to reverse retinal degeneration of T17M Retina
Yogesh Bhootada, Clark Gully, Marina S. Gorbatyuk. Vision Science, University of Alabama at Birmingham, Birmingham, AL.
Purpose: Class I & II rhodopsin gene (*RHO*) mutations cause endoplasmic reticulum (ER) stress that in turn activates the unfolded protein response (UPR) and results in cell death. Previously, we have shown that down regulation of a downstream mediator of the PERK branch, ATF4, completely rescues one-month-old degenerating retinas expressing the **T17M rhodopsin protein**. We hypothesized that simultaneous down regulation of two PERK mediators, ATF4 and CHOP, would give long term preservation to autosomal dominant retinitis pigmentosa (ADRP) mutations as compared to a single ATF4 knockdown.
Methods: T17M RHO ATF4$^{+/-}$ (single knockdown), T17M RHO ATF4$^{+/-}$ CHOP$^{+/-}$ (double knockdown), T17M RHO ATF4$^{+/+}$ CHOP$^{+/+}$ (T17M) and C57Bl/6 mice were used in the study. All groups were subjected to electroretinogram (ERG) at postnatal (P) day 30, P90 and P180. Proteins were extracted from retina at P30 to perform western blot analysis.
Results: A single knockdown rescued vision of P30 T17M mice and resulted in significant retardation of retinal degeneration at P90. In single ATF4 knockdown animals, a- and b- wave amplitudes of the scotopic ERG were higher (215% and 131%, respectively) than T17M mice, and lower (55% and 24%, respectively) than C57BL6 mice. Double knockdown of the PERK mediators resulted in a higher therapeutic effect than was provided by a single knockdown. At P90, these mice had an even greater increase in amplitudes of a- and b- waves (359% and 151%, respectively). This effect was sustained, lasted at least 6 months and lead to a dramatic reduction in the rate of retinal degeneration. The a-and b-wave amplitudes in P180 animals were 55% and 80% respectively of those found in the wild type retinas suggesting a preservation of physiological function of T17M photoreceptors. The therapeutic mechanism of ATF4-deficeincy in P30 T17M retinas was found to be associated with attenuation of UPR markers pEIF2A, pATF6 and CHOP by 70%, 32% and 72% respectively. Interestingly, reduction of ATF4 produced a significant 2.5-fold increase in both mouse and human rhodopsin expression as compared to T17M retinas which demonstrated significant inhibition of rhodopsin expression machinery.
Conclusions: ATF4 and CHOP deficiency dramatically preserves a loss of photoreceptors and their physiological function in T17M RHO retina suggesting that the PERK branch could be a viable therapeutic target for the treatment of ADRP.
Commercial Relationships: Yogesh Bhootada, None; **Clark Gully**, None; **Marina S. Gorbatyuk**, None
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Program Number: 5410 **Poster Board Number:** A0259
Presentation Time: 8:30 AM–10:15 AM
Gene therapy With Self-complementary Recombinant Adeno-associated Virus in Models of Autosomal Dominant Retinitis Pigmentosa Caused by *RHO* Mutations
Brian P. Rossmiller, Haoyu Mao, Alfred S. Lewin. Genetics, University of Florida, Gainesville, FL.
Purpose: Retinitis pigmentosa is the leading hereditary cause of blindness with 30-40% of cases attributable to autosomal dominant retinitis pigmentosa (ADRP). ADRP arises from mutations in at least 24 known genes with 30% arising in the rhodopsin gene (*RHO*). Given the large heterogeneity of mutations in *RHO* leading to ADRP, we propose knocking down of endogenous RHO and replacing it with a “hardened” copy, or a RHO with nucleotide changes that preserve the amino acid sequence but decrease the efficiency of knock-down. Here we report the use of a self-complementary Adeno-associated virus (scAAV) serotype 8 (Y733F) to express a hardened human rhodopsin (h*RHO*) under the control of the human opsin proximal promoter (HOPS) and an H1 promoter driven shRNA.
Methods: Four different knock-down methods were tested, ribozyme (Rz) 407, Rz525, miRNA 301 and shRNA 301 against both the wild-type and hardened *RHO* target regions in HEK293 cells. The reduction in expression of luciferase was measured at 24 and 48 hours post transfection.
Mice were treated at postnatal day 5 or 15 using scAAV to deliver these RNA knockdown agents to mouse models of ADRP: *Rho* I307N and human RHO transgenic T17M and P23H. The I307N mouse model exhibits very slow degeneration under ambient light but is reduced in visual response to light by 50% in one week post exposure to 10,000 lux. Intravitreal injections were done using two constructs, h*RHO*+shRNA301 or h*RHO*+shRNA750 in one eye and a control AAV-HOPS-mCherry or sham injection in the other. The mice were followed using electroretinogram and optical coherence tomography.
Results: The knock-down results show shRNA301 and ribozyme 525 to cause the largest reduction of RHO mRNA. At one month post injection there was no statistically significant difference between T17M *or P23H RHO* eyes injected with AAV-h*RHO*-shRNA750 and the sham injected eyes.
Conclusions: We have generated a series of combination RNA knockdown and replacement AAV vectors that may be useful for the treatment of ADRP. At early time points, our tests of these specific vectors have not been conclusive. The injected mice will be followed for longer intervals and additional mice will be added to the study to determine if the difference in visual function of the experimentally treated eyes versus the control is statistically significant.
Commercial Relationships: Brian P. Rossmiller, None; **Haoyu Mao**, None; **Alfred S. Lewin**, None
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Program Number: 5411 **Poster Board Number:** A0260
Presentation Time: 8:30 AM–10:15 AM
Topically Administered PBN and Methyl-PBN inhibits the visual cycle and slows rhodopsin regeneration in mice and baboon eyes
Megan Stiles, Nawajes A. Mandal, Madeline Budda, Gary White, Roman Wolf, Richard S. Brush, Robert Floyd, Robert E. Anderson. Ophthalmology, University of Oklahoma Health Science Center, Oklahoma City, OK.

Purpose: A2E and related toxic molecules are part of lipofuscin found in the retinal pigment epithelial (RPE) cells in AMD eyes. A novel therapeutic approach for AMD involves slowing down the visual cycle, which could reduce the amount of A2E in the RPE. This can be accomplished by inhibiting RPE65, which produces 11-*cis*-retinal from all-*trans*-retinyl esters. We discovered that phenyl-N-tert-butylnitrone (PBN), a spin trap agent, protects the retina from light-induced retinal degeneration (LIRD). PBN injected in rats slowed the rate of rhodopsin regeneration and recovery consistent with a slowing of the visual cycle and protected the retina from LIRD. In this study, we tested 1) whether topically administered PBN and certain PBN-derivatives can slow the rate of the visual cycle in mouse eyes and 2) the effects of PBN and Methyl-PBN (Me-PBN) in baboon eyes.
Methods: An eye drop formulation was used for topical application of PBN and its derivatives. To measure the effect of PBN on rhodopsin regeneration, light-adapted mice were given drops of PBN solution or vehicle followed by dark adaptation for 1 hour to recover bleachable rhodopsin. Eyes were harvested and rhodopsin levels measured spectrophotometrically. We developed various PBN derivatives, which were also tested under the same conditions as PBN. PBN and one of its derivatives, Me-PBN were topically applied to light-adapted baboon eyes followed by 1 hour dark adaptation to recover bleachable rhodopsin. Pieces of retina were harvested and used for rhodopsin measurement. The levels of PBN and Me-PBN in RPE-choroid tissue were measured by mass spectrometry.
Results: One hour dark adaptation resulted in 75-80% recovery of bleachable rhodopsin in control/vehicle mice. Eye drops containing 10% PBN inhibited (50-70%) the regeneration of bleachable rhodopsin. Among PBN derivatives, 5% Me-PBN was most effective, inhibiting the regeneration of bleachable rhodopsin by 70%. PBN and Me-PBN were then tested in anesthetized baboon eyes. PBN inhibited the visual cycle by 40%, whereas Me-PBN inhibited the cycle by 70% in baboons.
Conclusions: We conclude that topically applied PBN and its derivative Me-PBN can reach the target tissue and slow the rate of rhodopsin regeneration and therefore the visual cycle in both mouse and baboon eyes. PBN and its derivatives can potentially be developed as preventative treatment in human AMD.
Commercial Relationships: Megan Stiles, 13/775,771 (P); **Nawajes A. Mandal**, 13/775,771 (P); **Madeline Budda**, None; **Gary White**, 13/775,771 (P); **Roman Wolf**, None; **Richard S. Brush**, 13/775,771 (P); **Robert Floyd**, None; **Robert E. Anderson**, 13/775,771 (P)
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Program Number: 5412 **Poster Board Number:** A0261
Presentation Time: 8:30 AM–10:15 AM

PRPF31 delivery onto the retina using a nanoparticle system improves the visual acuity in a mouse model of retinal degeneration caused by A216P mutation in *PRPF31* gene

Berta De la Cerda[1], Francisco J. Diaz-Corrales[1], Lourdes Valdes-Sanchez[1], Ana Aramburu[1], Daniel Rodriguez-Martinez[1], Begoña Seijo[2], Andrea Pensado[2], Alejandro Sanchez-Barreiro[2], Shom S. Bhattacharya[1]. [1]Cell Therapy, CABIMER, Seville, Spain; [2]Department of Pharmacy and Pharmaceutical Technology, Faculty of Pharmacy, University of Santiago de Compostela, Santiago de Compostela, Spain.

Purpose: Very few therapeutical options exist today for retinal degenerative diseases. We used *Prpf31* $^{A216P/+}$ (KI) mice (Bujakowska et al. IOVS 2009) as a mouse model for inherited retinal disease to pre-clinically test the therapeutic activity of nanoparticle delivery of the *PRPF31* gene as a treatment for the haploinsuficiency caused by the mutation. The animal model used in our study harbors the human mutation A216P in *PRPF31* gene, which is known to cause retinitis pigmentosa (Vithana et al. Mol. Cell 2001). Mutations in this gene are also known to produce juvenile macular degeneration (Lu et al. PLOS one 2013).
Methods: Six-month-old C57BL/*6J* mice and WT littermates were anesthetized before the sub-retinal injection of 1μL of nanoparticles containing 200ng of plasmid for the expression of PRPF31 (Number of eyes, N=8) or GFP (N=6) under CMV promoter (pEGFP-N1, Clontech). Control animals were injected with 1μL of 5% glucose (N=6). One month after treatment, animals were subjected to optomotor test (OT), setting 6 frequencies from 0.031 to 0.272 cycles/degree (c/d) and 100%, 75% and 50% contrast sensitivity. Retinal structure and thickness were tested using optical coherence tomography (OCT).
Results: Statistically significant improvement in visual acuity was found in KI treated (*PRPF31*-injected) mice at 100% contrast at every frequency tested (p=0.02 at 0.272 c/d), showing a positive OT response in 75±2.8% of the cases in WT control (glucose-injected) animals and 68.5±8.6 in treated KI ones, while untreated KI (GFP -injected) mice showed only 33.3±4.4% of positive OT responses. Positive responses in KI treated animals decreased alongside with decreasing contrast, but still improved compared to untreated mutant mice. OCT measure of retinal thickness for WT control mice was 216.6±6.9 μm, for KI untreated mice was 198.5±1.9 μm and for treated KI mice was 211.9±13.5 μm. Thickness of the retina was significantly increased comparing KI treated and untreated samples (p=0.04).
Conclusions: Nanoparticle -driven delivery of the *PRPF31* gene to the subretinal space of mice has a therapeutical effect on the spatial vision (visual acuity and contrast sensitivity) and on the retinal thickness of the Prpf31 $^{A216P/+}$ mouse model.
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