PRO RETINA – FOUNDATION

Pro Retina – Stiftung
zur Verhütung von Blindheit

18th PRO RETINA
RESEARCH-COLLOQUIUM POTSDAM

Conference Report | April 12 – 13, 2024

Retinal Degeneration

Advancing Retinal Research

Genetics, Disease Modeling, and Therapy

Gefördert durch

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PRO RETINA Deutschland e. V. & The Pro Retina – Foundation for Prevention of Blindness

Who we are

Established in 1977 as the "Deutsche Retinitis Pigmentosa-Vereinigung," PRO RETINA Deutschland e. V. is a patient organization founded by individuals and their families seeking to organize assistance for themselves. The organization's constitution outlines four primary objectives: actively supporting research, providing psycho-social guidance to members, raising public awareness, and advocating for patient interests in politics, society, and the healthcare system.

Each member has the opportunity to affiliate with one of the 60 regional groups located throughout Germany. As of 2024, PRO RETINA Deutschland e. V. boasts a membership exceeding 6,500 individuals. The Board, counselors, leaders of regional groups, and all active members operate on a non-profit basis. Administrative support is provided by staff at our offices in Bonn, Germany (www.pro-retina.de).

What we do in research

In the realm of research funding, the Pro Retina Foundation has assumed a leading role in the prevention of blindness since 1996.

From its inception, we've cultivated a robust network comprising researchers and ophthalmologists, fostering collaborative information exchange and guidance. We provide direct financial support to research projects, having allocated over four million Euros since our inception. Additionally, we proactively initiate and contribute to research projects and therapeutic trials.

Annually, we confer two research awards, and facilitate national and international seminars and conferences on pertinent subjects. Furthermore, we sponsor Ph.D. grants to stimulate research activities and cultivate connections among researchers.

Our endeavors benefit from the guidance of both a Scientific and Medical Advisory Board and a Working Group on Clinical Issues. This Working Group comprises scientists from diverse medical and relevant disciplines.

Our primary aim is to ensure sustained support for research activities, whether by funding the development of new projects or underwriting the initial phases of significant endeavors.

We aspire to bolster the foundation's capital to a minimum of five million Euros, creating a dependable funding stream for research, independent of fluctuating donation income.

We affirm our commitment to exclusively dedicating the foundation's benefits to the research of retinal diseases, with the overarching goal of advancing therapies for patients.

PROGRAM

Friday, April 12, 2024

13:00 – 13:05 **Welcome remarks**
Franz Badura (Amberg, Germany)

13:05 – 14:30 **SESSION 1** **Chair: Antje Grosche**

SELECTED POSTER PRESENTATIONS
Eight abstracts to be selected

14:30 – 15:10 **Keynote lecture**
Kapil Bharti (Bethesda, USA)
Developing stem cell-based therapies for degenerative diseases of the eye

15:10 – 16:15 **Coffee break with scientific chit-chat**

16:15 – 17:30 **SESSION 2** **Chair: Peter Charbel-Issa**

KNOW YOUR GENE

16:15 – 16:40 **Maximilian Gerhard (Munich, Germany)**
Gene therapy in clinical practice

16:40 – 17:05 **Klaus Rüther (Berlin, Germany)**
Genetic diagnostics in retinal dystrophies – benefit for the patients

17:05 – 17:30 **Avril Daly (Dublin, Ireland)**
Retina International
Know your gene – the patient perspective

17:30 **Dinner**

19:00 – 19:30 **EVENING LECTURE**

Christine Curcio (Birmingham, USA)
Leveraging photoreceptor topography for deposit-driven AMD

19:30 – open **Swingin' poster session**

PROGRAM

Saturday, April 13, 2024

09:00 – 10:40 **SESSION 3** **Chair: Thomas Langmann**

UPDATES ON RPE IN RETINAL DEGENERATION

09:00 – 09:25 **Achim Göpferich (Regensburg, Germany)**
Nanoparticles for intraocular drug delivery

09:25 – 09:50 **Christelle Monville (Corbeil-Essonnes, France)**
Phase i/ii open-label study of implantation into one eye of hESC-derived RPE in patients with retinitis pigmentosa due to monogenic mutation: first safety results

09:50 – 10:15 **Peter Charbel-Issa (Oxford, UK)**
Novel tools to image the RPE in patients

10:15 – 10:40 **Anu Kauppinen (Kuopio, Finland)**
Antimycin A-induced mitochondrial dysfunction regulates inflammasome signaling in human retinal pigment epithelial cells

10:40 – 11:15 **Coffee break**

11:15 – 12:55 **SESSION 4** **Chair: Marius Ader**

RETINAL ORGANOIDS IN DEVELOPMENT, DISEASE AND THERAPY

11:15 – 11:40 **Volker Busskamp (Bonn, Germany)**
Generating vascularized human retinal organoids

11:40 – 12:05 **Magdalena Renner (Basel, Switzerland)**
High throughput retinal organoids for therapy development

12:05 – 12:30 **Majlinda Lako (Newcastle, UK)**
Retinal organoids: a window into development, disease and drug discovery

12:30 – 12:55 **Verena Hübschmann (Klosterneuburg, Austria)**
The impact of human microglia in retinal organoid development

12:55 – 13:00 **Concluding remarks**

13:00 **Farewell Lunch and end of meeting**

POSTER ABSTRACTS

Halting vcp activity as novel neuroprotective strategy against Retinal Degeneration in porcine and human models

Ana Cristina Almansa Garcia[1,2], Bowen Cao[1,2], Angela Armento[1], Merve Sen[1], Ellen Kilger[1], Anne-Sophie Petremann Dumé[1], Sylvia Bolz[1], Christine Henes[1], Blanca Arango-Gonzalez[1] and Marius Ueffing[1]

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Purpose: In our prior study, we found that inhibiting valosin-containing protein (VCP) pharmacologically effectively halted the degeneration of photoreceptors (PRs) in rodent models of retinitis pigmentosa by mitigating endoplasmic reticulum (ER) stress and enhancing energy homeostasis. Given the close interplay between oxidative stress and ER stress in retinal degeneration, our investigation extended to assessing the impact of VCP inhibition in cone-rich porcine retinal explants co-cultured with human iPSC-RPE cells. We examined this impact both in the presence and absence of induced oxidative stress and further examined human retinal explants to evaluate neuroprotection of photoreceptors and potential drug-induced toxicity.

Methods: Retinal explants obtained from the cone-rich visual streak of enucleated porcine eyes, in combination with human iPCS-RPE cells, and explants from two human donor eyes enucleated for uveal melanoma removal, were cultured in a medium-air interphase for up to 8 days. Inhibition of VCP was achieved using ML240. In the porcine retina, oxidative stress was induced by treating with hydroquinone (HQ) alone or in combination with ML240. Rhodopsin, iba-1, cone opsins, and cone arrestin immunostaining, along with TUNEL assay and outer nuclear layer cell row quantification, were used to assess retinal morphology, cell death, and cone PR survival.

Results: In the porcine retina, administration of HQ led to a decrease in the number of rows of nuclei in the outer layer, a shortening of PR outer segments, and a decline in the population of cone PRs. VCP inhibition by ML240 protected against HQ-induced cone degeneration. Moreover, VCP inhibition in human retinal explants showed a trend toward more cones and longer outer segments in the ML240-treated retinas. Equally important, ML240 treatment exhibited no evidence of cytotoxicity compared to the controls after 4 and 8 days in vitro. After 8 days of ML240 treatment *in vitro,* we observed decreased microglia cell activation and -migration to the outer retina, indicating a reduction of inflammation compared to the vehicle control.

Conclusions: Pharmacological VCP inhibition demonstrates a protective effect on cone photoreceptors, shielding them from degeneration induced by hydroquinone in a co-culture model of porcine retinal explants/iPSC-RPE cells as well as in human retinal organotypic cultures. The observed neuroprotection, combined with the absence of any detectable cytotoxic side effects, provides compelling support for exploring VCP inhibition as a prospective therapeutic strategy for human retinal disorders.

Combined effects of complement sources and Complement Factor H (CFH) Y402H polymorphism on the phenotype of iPSC-RPE cells

Angela Armento[1], Inga Sonntag[1], Merve Sen[1], Simon J Clark[1], Rosario Fernandez-Godino[2], Berta de la Cerda[3], Marius Ueffing[1]

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[2] Fundacion MEDINA, Granada, Spain
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Purpose: Complement system activation and genetic variants in complement system genes have been strongly associated with Age-related macular degeneration (AMD), a degenerative disease of the macula, leading cause of blindness in the elderly population. One of the main genetic risks is the Y402H polymorphism in the Complement Factor H gene (*CFH*/FH), an inhibitor of complement system activation. In our previous work, we showed that FH loss in RPE cells disturbs cell homeostasis. In this study, we investigate the impact of the AMD risk polymorphism Y402H on iPSC-RPE cells stress response.

Methods: Following differentiation of iPSC cells into mature RPE cells, we investigated the phenotype of iPSC-RPE cells carrying *CFH* 402Y (low risk) or 402H (high risk). Oxidative stress was induced in RPE cells via treatment with Hydroquinone (HQ), a component of cigarette smoke. RPE cells were additionally exposed the to normal human serum (NHS), inactivated NHS (iNHS) and C3b. The response to stress was assessed *in vitro* by cytotoxicity, oxidative stress and caspase activity assay (mean±SEM, n>4).

Results: RPE cells *CFH* 402H showed increased cytotoxicity, caspase activation and H_2O_2 levels compared to *CFH* 402Y when exposed to HQ. The exposure of RPE cells to NHS, iNHS or C3b had no impact on cell cytotoxicity, independent of the RPE genotype. Caspase activation was not influenced by complement sources in RPE cells *CFH* 402Y, while RPE cells *CFH* 402H showed an even higher activation when exposed to NHS. NHS exposure led to a general increase in H_2O_2 levels.

Conclusions: Our data support the hypothesis that FH, beside its systemic role, also acts as a modulator of RPE cells homeostasis. As a result, *CFH* 402H RPE cells are unable to properly respond to stress insult. In addition, it appears that extracellular complement sources may amplify the damage caused by FH 402H variant. These findings help to elucidate the complex function of FH in the RPE, highlighting novel possible non-canonical functions of FH in AMD pathology.

Cellular and molecular characterization of retinal degeneration in a novel mouse model of cone-rod dystrophies

Avesani A[1], Dal Cortivo G[1], Marino V[1], Biasi A[1], Asteriti S[1 2], Cangiano L[2], Targa G[1], Pigozzi A[1], Cambiaghi M[1], Dell'Orco D[1]

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Purpose: Cone dystrophies and Cone-Rod dystrophies (CORDs) are severe forms of inherited retinal diseases characterised by progressive degeneration of photoreceptor cells that can lead to complete blindness. To date, no treatment is available to stop the progression of the disease. Photoreceptor viability is strictly dependent on the levels of the second messengers cGMP and Ca^{2+}, the intracellular concentrations of which are finely regulated by guanylate cyclase-activating proteins (GCAPs) and their GC targets. Several mutations in the genes coding for GCAPs have been associated with autosomal dominant CORDs. Among these, p(E111V) GCAP1 variant has shown to lead to increased intracellular Ca^{2+} and cGMP levels [1,2] and recently, we confirmed that the delivery of E111V-GCAP1 protein in mice induces a disease-like electrophysiological phenotype [3], consistent with constitutive cGMP synthesis and increased Ca^{2+} level. In this work, we investigated the role of the E111V-GCAP1 and its involvement in the CORD-related phenotypes in a newly developed knock-in mouse model.

Methods: Both heterozygous and homozygous CORD mouse models and wildtype C57Bl/6 J mice of both sexes were analysed at different time points in order to investigate: i) morphological changes in the thickness ratio between the outer nuclear layer (ONL) and the inner nuclear layer (INL), ii) the expression of specific retinal proteins iii) and behavioral differences. For each point, mice were anaesthetised and euthanised via cervical dislocation and their retinas were extracted through a corneal incision. Then retina sections were processed for Immunofluorescence technique to analyse morphological changes and from the same eyes retinas were also homogenised for RNA extraction to measure the expression levels of GCAP1 and GCAP2. In detail, RNA of each animal was firstly used for cDNA synthesis using a reverse transcription kit and then Real-time PCR amplification was performed. In addition, we subjected each mouse genotype to a comprehensive behavioral test battery. To assess the potential of liposome-mediated delivery of GCAP1 in a mouse model, the human recombinant protein was encapsulated into a liposome with a lipid composition similar to that of the disks present in the Rod Outer Segment (ROS-like).

Results: We present the preliminary results on the morphological and behavioral characterisation of disease progression in both hetero and homozygosis mice with respect to the wildtype and the possible involvement of GCAP2 isoform in compensating for the dysregulation induced by the disease-associated E111V-GCAP1. In addition, the possibility of using direct or liposome-mediated administration of recombinant human GCAP1 to modulate the phototransduction cascade in mouse rods is presented.

Conclusion: In this work, we characterised for the first time a novel both heterozygous and homozygous CORD mouse model and the time course of retinal degeneration. In addition, we show how the efficient delivery of functional recombinant WT GCAP1, either with or without the use of liposomes, could be promising for the treatment of retinal diseases.

[1] Marino V et al. (2018) Hum Mol Genet. 27(24), 4204-4217
[2] Dell'Orco D et al. (2019) Sci Rep. 9(1), 20105
[3] Asteriti S et al. (2023) Cell Mol Life Sci. 80(12), 371

Unveiling the limits: Gene therapy of retinitis pigmentosa cannot restore metabolic reprogramming

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Purpose: Retinitis pigmentosa (RP) is a hereditary retinal disease that leads to vision loss due to progressive degeneration of photoreceptors. The best hope for a cure for RP lies in gene therapy. However, data from clinical trials expose some sustainability concerns. Our goal is to define how the proteome changes as RP disease progresses, and to identify which changes can be reversed, halted, or not halted by gene therapy. We expect to identify novel markers of disease progression and new potential therapeutic targets to increase efficacy and sustainability of gene therapy.

Methods: We used an RP gene therapy mouse model in which a floxed STOP cassette in intron 1 of the phosphodiesterase 6b (Pde6b) gene prevents PDE6B-expression and, thus, rod function. The floxed STOP cassette can be removed using Cre, leading to restoration of PDE6B. This Cre-driven genetic rescue represents an idealized gene therapy scenario, where all rods are rescued. The mutant mice were treated (tamoxifen injected) at 4 weeks of age, when approximately 30 % of the rods have died. At 8 weeks of age, retinas were analysed by label-free liquid chromatography-tandem mass spectrometry-based proteomics (vs. untreated and WT control retinas). Dysregulated proteins were validated by RT-qPCR, immunoblot, and immunohistochemistry. Metabolic phenotypes were quantified by assaying metabolites.

Results: The bioinformatics analysis of the proteomics data identified dysregulated proteins and pathways associated with metabolism in RP retinas. For example, we found decreased levels of glycolytic enzymes (eg, PKM2) and increased expression of oxidative phosphorylation (OXPHOS) enzymes (eg, COX7A1, COX7B, and COX7C) in RP retinas (vs WT). These changes were accompanied by reduced lactate and increased ATP levels. Immunoblotting of OXPHOS complexes I-V confirmed the upregulation of the mitochondrial respiratory chain in RP retinas. Interestingly, these changes were also observed in treated retinas, suggesting that rescued retinas have not achieved metabolic homeostasis.

Conclusion: We performed an extensive analysis of WT, mutant and treated RP retinas, to better understand the molecular mechanisms underlying RP disease progression and therapeutic efficacy. Our findings demonstrate a transition from aerobic glycolysis to OXPHOS in mutant retinas, suggesting that metabolic demands differ in RP. These metabolic changes were not rescued by treatment and could be a critical determinant of the long-term success of gene therapy for RP.

Connecting the dots: KCNE2 and TRPM1 signaling interactions at the rod on-bipolar cell synapse

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Background/Purpose: Voltage-gated potassium channel subfamily E member 2 (KCNE2) is an ancillary subunit that is known to interact with potassium variety of six transmembrane domain cation channels (Kv α subunits). It plays a crucial role in shaping channel properties, influencing subunit composition, pharmacology, and trafficking. In a recent study we discovered the expression of KCNE2 at the postsynaptic membrane of rod-ON-bipolar cell synapses, where a complex and incompletely understood signaling machinery containing a calcium-permeable cation channel TRPM1 is in place and integral for the transmission of visual information. While a mutation in this channel leads to congenital stationary night blindness, the specific role of KCNE2 in this context needs to be defined.

Methods: To understand the function of KCNE2 at the ON-bipolar cell synapse and identify its interaction partner, we have established an ON-bipolar cell synapse-specific knockout mouse line for *KCNE2.* This was subsequently characterized by using immunohistochemical, western blot, and qualitative reverse transcriptase PCR approaches. In parallel, we performed single-cell RNA sequencing analyses to identify candidate interaction partners for Kcne2 and carried out immunocytochemical and biochemical experiments in heterologous expression systems to characterize these further.

Results: Kcne2 expression is selectively lost at the postsynaptic membrane of the ON-bipolar cell synapse in the novel mouse line. Single-cell RNA sequencing analyses indicate a strong correlation between KCNE2 and TRPM1 specifically in ON-bipolar cells at the transcriptomic level. Consistently in real-time quantitative PCR, TRPM1 is down-regulated in the *KCNE2* knockout mouse. Simultaneously, in a heterologous expression system, we observed a drastic reduction in the TRPM1 signal when co-transfected with KCNE2 for 48 hours through immunocytochemistry. Additionally, a time-point experiment was performed over a course of 48 hours to confirm the reduction of TRPM1 at the protein level when KCNE2 was transfected.

Conclusion: This study provides the first evidence of KCNE2 may interact with TRPM1, potentially connecting the missing dots in the TRPM1 signaling machinery at the rod-ON-bipolar cell synapses.

Inhibition of the IL-6 signalling pathway to reduce rAAV induced inflammation

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Introduction: Adeno-associated viruses (AAV) are the most widely used vector platform for ocular gene therapy. Apart from the risks of the invasive procedure, there is now increasing evidence that gene therapy-associated uveitis (GTAU) can develop following intravitreal or subretinal administration of the vector. The significance of this GTAU is important for therapeutic efficacy and safety. The control of the responsible immunomodulators such as Interleukin-6 (IL-6), a cytokine involved in the regulation of immune responses and inflammation. In order to develop appropriate treatment strategies to control inflammation in gene therapy recipients, drugs like tocilizumab, an IL-6R inhibitor, can be used to modulate the IL-6 pathway in hopes of improving the aforementioned conditions.

Methods: Biocompatibility of tocilizumab in hTERT and ARPE-19 cells was tested by determining cell viability, through MTS assay, and cell number, through crystal violet assay. To induce IL-6 dependent inflammation, porcine retinal explants were incubated for 48h with AAV5 and AAV9 ($1x10^{10}$) vectors. Tocilizumab (50 µg/ml) was added in parallel to rescue the induced inflammation. Molecular effects were tested using qRT-PCR by detecting the following markers, *Il-6, IL-1β, TNFα,* and *NLRP3.*

Results: 5 µg/ml-50 µg/ml Tocilizumab exhibited high biocompatibility (85-108% cell viability relative to control, 82-126% cell number relative to control) in hTERT and ARPE-19 cells. These effects were confirmed with qRT-PCR, where expression of *IL-6, IL-1β, TNFα, and NLRP3* was not significantly altered due to the treatment of 5-50 µg/ml Tocilizumab in retinal explants after 24h. In contrast, treatment with $5x10^{10}$ rAAVs increased *IL-6* gene expression in AAV5 and AAV9. Furthermore, retinal explants exposed to rAAV9 were simultaneously treated with tocilizumab followed by qRT-PCR after 48h to measure gene expression of *IL-6, IL-1β, TNFα,* and *NLRP3*. With tocilizumab treatment *IL-6* gene expression returned to levels similar to the negative control.

Conclusion: Tocilizumab showed satisfactory levels of biocompatibility in *in vitro* and *ex vivo* models. Treatment with rAAV5 and rAAV9 induced an IL-6 pro-inflammatory response in retinal explants thereby simulating the side-effects of ocular gene therapy. Upon administration of tocilizumab *IL-6* gene expression decreased dramatically. Therefore, parallel administration of tocilizumab is a potential treatment option for GTAU.

Pharmacological inhibition or genetic inactivation of ERAD effector VCP suppresses retinal degeneration in a knock-in mouse model caused by isoleucine 255/256 deletion in the rhodopsin gene

*Bowen Cao[1,2], Johanna Valentina Dahlen[2], Ana-Cristina Almansa-Garcia[1,2], Merve Sen[1], Tina Beyer[1], Ellen Kilger[1], Francois Paquet Durand[1], Blanca Arango-Gonzalez[1,] * and Marius Ueffing[1,] **

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Purpose: This study aims to elucidate the pathomechanisms underlying the autosomal dominant retinitis pigmentosa (adRP) caused by a deletion in the rhodopsin gene, leading to loss of one isoleucine (I) at position 255/256 ($Rho^{\Delta I255}$/$Rho^{\Delta I256}$), and study the effects of pharmacological inhibition or silencing the expression of valosin-containing protein (VCP), an endoplasmic reticulum-associated degradation (ERAD) effector.

Methods: HEK293 cells were transfected with plasmids encoding EGFP-tagged Rho^{WT} or $Rho^{\Delta I255}$. Rhodopsin protein (RHO) degradation along the ERAD pathway was analyzed via immunostaining. Following treatment of transfected cells with VCP inhibitor ML240, RHO levels were analyzed by western blot. Neuroprotective effects of VCP pharmacological inhibition using ML240 or silencing via VCP siRNA were evaluated in heterozygous $Rho^{\Delta I255}$ ($Rho^{\Delta I255/+}$) mouse retinal explants. TUNEL staining was used to detect photoreceptor (PR) cell death in the retinal outer nuclear layer (ONL), while immunostaining was used to assess RHO localization and expression. Gliosis and retinal microglial activation were evaluated before and after VCP inhibition using antibodies against GFAP and Iba-1.

Results: Unlike normal RHO, $RHO^{\Delta I255}$ formed perinuclear aggregates and trapped RHO^{WT} in transfected cells. $RHO^{\Delta I255}$ colocalized with ERAD markers calnexin, VCP, ubiquitin, and PSMB5. Inhibition of VCP impaired the clearance of $RHO^{\Delta I255}$ by ERAD, increasing the generation of high molecular weight aggregates. In $Rho^{\Delta I255/+}$ retinal explants, VCP inhibition and VCP silencing reduced the number of TUNEL-positive cells in the ONL (ML240 0.38 ± 0.15, control 1.69 ± 0.43; P<0.01/ VCP siRNA 0.83 ± 0.16, scrambled siRNA 3.38 ± 1.25; P<0.01), and improved the correct RHO targeting, represented by the OS thickness (ML240 10.51 ± 3.80, control 5.57± 1.19; P<0.001/ VCP siRNA 26.63 ± 4.83, scrambled siRNA 15.20 ± 2.59; P<0.001). Moreover, inhibition of VCP reduced both GFAP staining and microglial migration in the ONL.

Conclusions: Mutant $RHO^{\Delta I255}$ traps normal RHO at the ER and is degraded by ERAD with the requirement of VCP. Pharmacological or genetic inhibition of VCP releases RHO, rescues PR from cell death, enhances correct RHO distribution in the OS, and reduces microglial activation.

Novel mutation-independent gAON approach to treat RHO-linked autosomal dominant Retinitis Pigmentosa

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Introduction: Retinitis pigmentosa (RP) is the most prevalent form of inherited retinal disease, affecting ~1 in 5000 people worldwide. Within the spectrum of RP, autosomal dominant RP (adRP) accounts for ~25-30% of all RP cases. Mutations in the *RHO* gene represent the most common causative factor, mostly acting as gain-of-function or dominant-negative mutations. Because of these underlying pathological effects, gene supplementation gene therapy is not a viable therapeutic option. Consequently, main research efforts towards gene-based therapies for *RHO*-associated adRP have focused on "knockout and replace strategies", which combine the knockout or knockdown of the endogenous *RHO* gene (both the mutant and the wildtype allele) with the simultaneous delivery of a knockout/knockdown-resistant wildtype *RHO* cDNA. However, while promising pre-clinical results have been obtained in animal models, a major unsolved hurdle still remains, which is controlling the expression level of the *RHO* transgene in transduced cells. Even as little as 23% of RHO overexpression is retinotoxic in mice and copy number variants in wildtype *RHO* have now been reported as a novel cause of adRP. In contrast, reduced *RHO* gene dosage does not result in disease, as evidenced by early nonsense mutations, exhibiting a recessive inheritance pattern.

Methods: We developed a mutation-independent therapeutic strategy able to selectively lowers the amount of the mutant RHO protein through gapmer antisense oligonucleotides (gAONs) targeting. By leveraging on a benign single nucleotide polymorphism (SNP - G/A), frequently found in heterozygous state (e.g. 30% in our patient cohort), gAONs are designed to specifically target either SNP-containing *RHO* transcript. Consequently, as per design, transcripts containing the mutant *RHO* sequence in *cis* with the targeted SNP will be destructed. Thirty-two gAONs were screened for potency and specificity, using fluorescent reporter cells stably expressing RHO at high level.

Results: Screening of gAONs led to the identification of candidates able to knock-down RHO protein expression between 35% to 65%, while also exhibiting favorable allele preference. Next phase includes the validation of lead gAONs in patient-derived retinal organoids and safety profile assessment.

Conclusions: By leveraging on a therapeutic modality already approved for clinical use, our approach holds great potential for advancing effective treatments for a great proportion of RHO-linked adRP patients.

Analysis of galectins-1,-3 and -9 during the inflammatory response driven by retinal neurodegeneration

Nundehui Díaz-Lezama and Susanne F. Koch

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Purpose: Retinitis pigmentosa (RP) is often driven by mutations in the light-sensitive rod photoreceptors, which trigger their death and, secondarily, the death of cone photoreceptors. While the mechanisms driving the "bystander" cone degeneration in RP have not been defined, inflammation has long been implicated. Previous studies have shown that galectins are key players in neuroinflammation and might be potential therapeutic targets for neurodegenerative disorders. Thus, our goal was to determine the expression of galectins in healthy and RP retinas and to probe a link between galectins, inflammation and photoreceptor degeneration.

Methods: We characterized the inflammatory response, photoreceptor degeneration and galectins in retinas from a *Pde6b*-deficient RP mouse model by proteomic profiling, immunohistochemistry (IHC) and immunoblots.

Results: Photoreceptor degeneration in our RP mouse model was accompanied by microglia and Müller glial cell activation and increased retinal expression and activity of inflammatory key regulators. Our proteomics analysis of Müller glial cells revealed 4 different galectins: Galectin-1,-3,-3bp, and -9. Galectin-1, -3bp and -9 were significantly upregulated in Müller glial cells from our RP mice (vs WT), whereas Galectin-3 was not altered. We confirmed these results by IHC and immunoblotting. In addition, we observed subpopulations of microglia/macrophages with high and low levels of galectin-3 expression in the RPE.

Conclusions: Our data demonstrate a specific expression pattern of galectins-1, 3, -3bp and -9 during RP disease progression, suggesting that galectins undergo different stages of activation that are associated with either an acute or chronic inflammatory response.

Retinal microglia and their immunotoxic effects in AAV-based gene therapy

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[1] Department of Ophthalmology, Laboratory of Experimental Immunology of the Eye, University of Cologne
[2] Center for Molecular Medicine Cologne (CMMC), University of Cologne

Purpose: In recent years, gene therapy targeting inherited retinal diseases (IRDs) using adeno-associated virus (AAV) as vector have shown immense promise. After Luxturna®, an AAV-based treatment for a biallelic mutation in the RPE65 gene, gained approval by the FDA in 2017, multiple clinical trials have been started for treatment of other IRDs using various AAV-based approaches. However, despite the relatively low immunogenic potential of AAVs, and the immune privilege of the eye, numerous reports of inflammation after treatment show that AAV vectors are recognized as foreign invaders and elicit host-cell responses. Microglia, as the resident immune cells of the retina, are highly involved in these inflammatory processes. Our preliminary data together with data from other research groups have indicated that resident microglia are early responders to locally injected retinal AAVs, however, a comprehensive analysis in adult animal models is still missing. A deeper understanding of immune activation during retinal AAV gene therapy, as well as what factors determine the immunogenicity of the vector, is crucial to ensure the safety of these approaches.
Our hypothesis is that retinal immune cells such as microglia are activated very early in response to AAV and that their inflammatory responses negatively affect the success of ocular gene therapy. This hypothesis also considers the aspect that retinal microglia maintain a chronic pro-inflammatory state in the already degenerating retina and that innate immune memory effects might thus induce microglia to react more strongly against AAV in the future.

Methods: Two AAV capsids will be packed either with a non-coding stuffer sequence or the full-length coding sequence of murine Fam161a. Cx3cr1-GFP$^{+/+}$ reporter mice will be intravitreally injected with AAVs at three different titres and analysed at pre-determined time points post injection. In a comprehensive analysis combining in vivo retinal imaging that will allow to identify GFP+ microglia in these reporter mice alongside assessing retinal structure with histological and molecular approaches, we will quantify different key parameters of microglial reactivity and inflammation with regard to capsid, titre and transgenes. Based on these findings, an experimental AAV2 vector-based gene augmentation therapy in Fam161a-deficient mice under conditions of potent immunomodulation will be done.

Single cell RNA sequencing reveals metabolic shift in Müller glial cell response upon TSPO knockout in the ischemic retina

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Background: Müller cells, the predominant macroglial in the retina, play a pivotal role in supporting neuronal metabolism and function. They are critical for regulating ion and water homeostasis, maintaining the blood-retinal barrier, and, together with microglia, controlling the tissue immune response. Remarkably resilient to damage, Müller cells respond to retinal injury and disease by changing their biochemical and physiological features through a process termed reactive gliosis. The Translocator protein 18 kDa (TSPO), an integral membrane protein of the outer mitochondrial membrane, facilitates cholesterol transport into the mitochondrial matrix - a rate-limiting step in steroid biosynthesis. In the normal neuroretina, TSPO is predominantly expressed in Müller glia and vascular cells, underscoring its significance in retinal health. Here, we investigated how Müller cell-specific TSPO knockout alters their transcriptomic landscape in response to ischemia at the single cell level.

Methods: A single cell suspension obtained from both WT and Müller cell-specific TSPO KO mouse retinae of control and ischemic eyes 7 days post injury was subjected to magnetic activated cell sorting (MACS), which enriched Müller cells using the CD29 surface marker. Single cell RNA sequencing was then performed and the data were analyzed in RStudio (version 4.3.2) with the Seurat package.

Results: We identified 24 clusters corresponding to 12 retinal cell types, with 7 clusters showing a Müller cell-specific expression signature. Focussing on the seven Müller cells clusters, we found an almost even distribution across genotypes. Notably, when comparing the amount of cells retrieved from different treatments, we observed more cells from ischemic than from control retinae. Further analysis of Müller cell markers, including glutamate synthetase, vimentin, Tgfb2, and GFAP, helped us identify three clusters revealing signs of gliosis. We discovered differentially expressed genes (DEGs) between control and ischemic conditions in Müller glial cells, with a notable upregulation of DEGs in TSPO knockout retinas in response to ischemia. Additionally, our examination of metabolic gene expression in Müller cells showed a decrease in normal TSPO knockout retinae compared to wild types. However, ischemic injury led to an increase in metabolic gene expression in TSPO knockout Müller cells, highlighting the impact of TSPO on the metabolic response of Müller glial to tissue damage.

Conclusion: Our findings show that TSPO knockout enhances the response of Müller cells to ischemia, as evidenced by upregulation of cell-specific markers. Remarkably, the expression profile of metabolic genes indicates compensatory mechanisms in TSPO KO Müller cells in response to ischemia.

Red light as a potential therapy for oxidative stress-based retinal diseases

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Background: With aging society, it is not only important to develop therapies against age-related diseases, but also to discover preventive methods which minimize the risk of these diseases. Long-wave red light (RL) increases ATP production and thus viability of retinal cells. Initial clinical studies for photo biomodulation therapy have indicated that RL can delay the progression of AMD. In contrast, blue light (BL) has a high photochemical energy that increases oxidative stress and may play a role in age-related macular degeneration (AMD). Based on our BL degeneration model, retinal explants were exposed to RL before/after BL exposure. The aim of this study was to evaluate whether RL exposure could minimize BL-induced damage and therefore serve as a therapeutic option.

Methods: Porcine retinal organ cultures were cultivated for 24 h and exposed to BL (15 mW/cm^2, 455 nm) and RL (10 mW/cm^2, 670 nm). Oxidative stress levels were measured via H_2O_2 assay. Caspase 3/7 activity and ATP levels were determined after 6/24 h. Cell death was analysed by TUNEL staining. Gene expression of cell-specific markers (*opsin*, *β-III-tubulin, PKCα*), and apoptosis markers (*TNF-α, p53, Bax/Bcl-2*) was determined using qRT-PCR. Protein levels of GFAP, HSP70 and NF-kB were evaluated by western blot and immunohistology.

Results: RL exposures led to decreased oxidative stress and caspase 3/7 activity in contrast to BL exposures, which led to increased oxidative stress, caspase 3/7 activity and TUNEL+ cells in retinal explants. Protein levels of GFAP (+249%) and HSP70, as well as gene expression levels of apoptosis markers (*TNF-α, Nf-κB, Bax/Bcl-2*) were enhanced. RL applied before or after BL exposure, led to a reduction of oxidative stress (-54%), caspase 3/7 activity, NF-kB (-68%) and GFAP (-65%) protein levels, TUNEL+ cells and *Bax/Bcl-2* gene expression (-38%). The gene expression of the cell markers *opsin*, *β-III-tubulin* and *PKCα* was rescued due to RL exposures.

Conclusion: Exposure to BL led to increased oxidative stress and cell death in retinal explants, thus simulating oxidative stress based retinal diseases like AMD. RL exposure was able to prevent or even reverse this damaging effect. These data therefore suggest that RL exposure can be used both preventively and as a therapy against oxidative stress in the retina.

EDSpliCE, a CRISPR-Cas strategy suitable for correcting pathogenic exonic variant in *ABCA4*

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Purpose: Bi-allelic pathogenic variants in ***ABCA4*** are causative of Stargardt disease (**STGD1**), an autosomal recessive retinal disorder, characterized by progressive loss of central vision and typically presenting during childhood. The mutational spectrum of *ABCA4* is diverse, with the **exonic c.768G>T variant** being a severe and frequent variant underlying STGD1. This variant results in mis-splicing by inducing a 35-nucleotide elongation of exon 6 retained in the mature mRNA transcript. This is due to the weakening of the canonical donor splice site and the presence of a strong cryptic splice donor site located 36 nucleotides downstream. Consequently, a frameshift occurs, leading to the formation of a truncated protein, p.(Leu257Valfs*17).

CRISPR/Cas-mediated genome editing has been shown to effectively correct splicing defects and restore proper protein translation, providing a potential and permanent solution for genetic defects caused by splicing-affecting variants. However, to date, no CRISPR/Cas-mediated strategy has been established to correct faulty splicing due to pathogenic variants located nearby to exon/intron boundaries. To tackle this issue, we utilized our novel gene editing platform namely **Enhanced-Deletion Splicing Correction Editing (EDSpliCE)** for the successful correction of the splicing defect caused by the c.768G>T variant in minigene assay experiments. **EDSpliCE enables directional and enhanced deletions at the targeted site.**

Methods: A mutant (MT) minigene plasmid containing the *ABCA4*:c.768G>T variant was cloned. The presence of pathogenic splicing for this variant was confirmed through minigene assay in HEK293T. The validated MT minigene was then co-transfected with one of four different single guide RNAs (sgRNA) and the Cas9-ortholog EDSpliCE. The percentage of correctly spliced transcripts was calculated through mRNA splicing analysis, followed by PCR reaction analysis using chip electrophoresis as a readout. Editing efficiency for the most promising sgRNAs was analyzed by targeted high-throughput sequencing (HTS).

Results: Correction of the c.768G>T-induced splicing defect by EDSpliCE yielded to 85% correct *ABCA4* transcript compared to limited rescue achieved with the parental wild-type Cas9-ortholog. HTS confirmed the generation of directional and enhanced deletion at the targeted sites.

Conclusions: Our results show that EDSpliCE effectively corrects the splicing defect caused by the c.768G>T variant. This positions EDSpliCE as a promising and advanced gene editing approach with significant potential for therapeutic applications, particularly in addressing splicing variants within *ABCA4* and beyond.

Fluorescence lifetime imaging microscopy (FLIM) of pigmented cells in neovascular age-related macular degeneration (AMD)

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Purpose: Melanotic cells are often found in fibrotic tissue in the outer retina in the course of late stage neovascular AMD. De novo synthesis of melanin or conversion of individual organelles in th context of epithelial-mesenchymal-transition (EMT) of the retinal pigment epithelium (RPE) are possible mechanisms in the formation of these pigmented lesions, but the pathogenesis and impact of these lesions is not yet fully understood. Fluorescence lifetime imaging microscopy (FLIM) enables to examine underlying fluorophores or groups of fluorophores in autofluorescent tissue. This study uses FLIM to examine these pigmented lesions and to further determine the origin of the pigment.

Methods: Macular cross sections of 5 human donors with late stage neovascular AMD (age 91.5 ± 2.1 yrs) were imaged using FLIM (confocal laser-scanning microscope, excitation 488 nm). Five sections of 5 human eyes with unremarkable macula (age 82.5 ± 0.7 yrs) served as controls. Mean fluorescence lifetimes of in-layer RPE, pigment in outer retinal fibrotic tissue, and pigment within the choroid were analyzed. Mann-Whitney-U tests were performed for significance analysis.

Results: A total of 115 regions with in-layer RPE or pigmented lesions could be FLIM imaged and analyzed. Relative to intact in-layer RPE of normal eyes (mean 0.48±0.03 n), melanotic cells in neovascular AMD eyes showed significantly prolonged fluorescence lifetimes (mean 0.52±0.04 ns; $p < 0.01$). However, melanotic cells in fibrotic lesions in the outer retina (0.48 ± 0.02 ns) were not significantly different to the intact RPE (0.5 ns ± 0.04 ns; $p = 0.5$). Relative to intact in-layer RPE of the normal eyes, melanotic cells of the choroid showed distinctly shorter lifetimes than intact RPE (0.22 ns ± 0.05 ns, $p<0.01$).

Conclusion: Fluorescence lifetimes indicate that melanotic cells in fibrotic scar tissue in neovascular AMD might originate from RPE cells. A further understanding of the transition of these cells might help to describe pathogenic mechanisms and to develop therapeutic strategies for RPE survival in AMD.

The impact of choriocapillaris integrity on RPE and photoreceptors

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Purpose: The choriocapillaris (CC) forms an extremely dense capillary network that consists of fenestrated capillaries with numerous fenestrations. The fenestrations are spanned by a diaphragm of radially oriented fibrils that substantially limit the passage of plasma proteins. Plasmalemma-vesicle associated protein (PLVAP) is the major constituent of diaphragms and the lack of PLVAP disrupts their formation. Here we analyzed the effect of an induced PLVAP-deficiency on CC, RPE and retina.

Methods: We used 4-weeks-old CAGG-Cre-ER/*Plvap*$^{fl/fl}$ mice and their *Plvap*$^{fl/fl}$ littermates of C57BL/6J background and induced PLVAP-deficiency *via* tamoxifen administration (5mg/ml 3x/d for 5 days). The mice were analyzed at ages of 12 weeks or 6 months. PLVAP synthesis was evaluated by Western blot analysis of CC-samples or kidney-samples, which served as reference. PLVAP-loss was visualized by CC flat-mounts stained against PLVAP and cluster of differentiation 31 (CD31) and quantified. CC-fenestration was quantified *via* transmission-electron-microscopy. RPE-morphology was analyzed and quantified by CC/RPE flat-mounts stained against PLVAP and the tight junction protein Zonula occludens-1. Viability and health of RPE and sensory retina was evaluated by TdT-mediated dUTP-biotin nick end labeling (TUNEL), morphometric analysis of retinal layers, and quantification of RPE-autofluorescence on sagittal sections. In addition, CC-network of 12-weeks- and 6-months-old wildtype mice was compared with CC flat-mounts stained against PLVAP and CD31.

Results: Western blot data confirmed that PLVAP-synthesis was significantly reduced to 30% in 12-weeks-old CAGG-Cre-ER/*Plvap*$^{fl/fl}$ mice. PLVAP-loss was concentrated on 14% of the central and 34% of the peripheral CC-network. The ratio of diaphragms/µm was not altered in the central CC while in the peripheral CC it was significantly reduced by 66%. Morphological and morphometric analysis showed no structural alterations of RPE or sensory retina. RPE-autofluorescence was increased by 35%. TUNEL-assay showed no increased cell death. Western blot data of kidney-samples showed no further increased PLVAP-deficiency between 6-months- (45%) and 12-weeks-old (27%) mice. However, in 6-months-old PLVAP-deficient mice no significant PLVAP-loss in the central and a 10% loss in the peripheral CC were detectable. Peripheral RPE-autofluorescence was further increased (59%), without increased cell death. Comparison of 12-weeks- and 6-months-old wildtype CC flat-mounts revealed a significant PLVAP-loss in central (17%) and peripheral (32%) CC caused by aging.

Conclusion: Moderate PLVAP- and diaphragm-loss has no harmful consequences for RPE and retina.

Mapping the landscape of Müller cells in human retinal tissue

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Lower vertebrate retinas exhibit regenerative potential through Müller cells, capable of generating glia and neurons by entering the cell cycle and transforming into a multipotent progenitor stem cell. While it has been generally accepted that Müller cells in mammals have lost their ability to divide, recent results contest this view.

Eyes of adult multi-organ donors without known ocular disease were removed prior to cardiac arrest and subjected to immunohistochemistry using mitotic, progenitor, and cell-type-specific markers after fixation. The experiments were designed to compare the periphery and central region of the retina.

The Ki-67 proliferation marker detected a significant amount of dividing cells. A subset of Ki- 67 positive cells expressed Pax6 protein, indicating their retinal origin. A fraction of dividing cells colocalized the Müller cell-specific Sox9 protein. Labeling with any of S100B or RLBP1 or DBI or Sox9, and Sox2, Pax6, and Vsx2 progenitor markers revealed co-expression. Distinct Müller cell clusters were identified.

To our knowledge, this study represents the first evidence of adult human Müller cell division in the original, intact, three-dimensional environment, without exogenous growth or stimulatory factors.
These findings raise the possibility that selective, vector-mediated transduction of Müller cells could be used to induce their division in a targeted and controlled manner. Based on the results of experiments in rodent models, it may be feasible in the future to replace lost retinal neurons by genetically reprogramming the generated cells.
A therapy based on this principle could apply to several degenerative retinal diseases.

Immunogenicity of recombinant AAVs in retinal gene therapy

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Inherited retinal diseases (IRD) represent a genetically heterogeneous group of rare blinding conditions, which is characterized by dysfunction or degeneration of photoreceptors, the retinal pigment epithelium (RPE) or the choroid leading to visual impairment or to a complete loss of vision. X-linked juvenile retinoschisis (XLRS) is a recessive degenerative disease of the central retina, triggered by mutations in the RS1 gene. RS1 encodes for the protein retinoschisin, which is responsible for cell adhesion and cell-cell interactions required for maintenance of retinal stability and synaptic integrity.
Gene therapies using adeno-associated viruses (AAVs) are among the most promising strategies to treat IRD. Although AAV vectors are considered to be safe, they are recognized as foreign invaders and elicit host-cell responses even in immune privileged areas such as the eye.
A deeper understanding of these immune processes in the retina is important for eliminating immune-related side effects and toxicity that negatively impact gene therapy efficacy. Microglia, the resident immune cells of the retina, are not only bystanders but can also trigger retinal degeneration and potentially serve as targets for therapy. Our preliminary data together with recent findings from collaborators indicate that microglia are early responders to locally injected AAVs. Beside the impact of virus titer on the immunogenicity of AAVs, capsid protein variants, driving promoters and the transgene product also seem to be decisive factors. In this project, we will perform an in-depth analysis of retinal microglia at different stages after AAV transduction in response to different titers, capsid variants, ubiquitous and endogenous promoters as well as vectors containing different transgenes.
Furthermore, microglia reactivity in response to different AAV vectors will be studied on a human-based retina-on-a-chip technology.
We also aim to perform an experimental gene therapy in retinoschisin (Rs1h)-deficient mice under conditions of microglia modulation using minocycline.
This study will not only help to decipher toxic mechanism and pinpoint triggers for harmful retinal immune responses but also help to elucidate whether immunomodulation is a beneficial approach for improving gene therapy efficacy and durability.

Metabolic and functional dynamics during RPE remodeling in retinitis pigmentosa

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Purpose: Retinal pigment epithelium (RPE) cells are essential for the phagocytosis of aged photoreceptor outer segments (POS), a process that is vital for photoreceptors susceptible to photo-oxidative damage. In retinitis pigmentosa (RP), genetic mutations lead to a degeneration of photoreceptors but changes in the neighboring RPE have not been well characterized. Therefore, we used an RP mouse model to analyze the structural, functional, and metabolic changes of the RPE during disease progression.

Methods: We investigated RPE structure and function in a *Pde6b*-deficient RP mouse model and compared it to wild-type. The functional assessment involved the analysis of *ex vivo* POS engulfment and endogenous POS uptake at the peak of phagocytic activity. Further, we analyzed metabolic changes by screening mRNA and proteins that play an important role in aerobic glycolysis and oxidative phosphorylation.

Results: At the early disease stages (pw 8), RPE cells had a mostly hexagonal, uniform shape and were similar to wild-type mice. Only at late disease stages (pw 30), RPE cells were elongated and irregular in cell size. Interestingly, these morphological changes are preceded by altered expression of metabolic key proteins – for example, RPE65, which is involved in the visual cycle, glucose transporters such as GLUT1 and GLUT4, and oxidative phosphorylation enzymes such as OPA1, a dynamin-related protein essential for the fusion of mitochondria, cytochrome c oxidase 5b and UQCRC11 (subunit of ubiquinol-cytochrome C reductase). Additionally, we also demonstrated that dysfunction in the RPE phagocytosis is visible before morphological changes.

Conclusion: Our data suggest that mitochondrial dysfunction in RPE cells leads to RPE remodeling and highlights the importance of metabolic homeostasis in maintaining a well-functioning system between RPE and photoreceptors. Given the pivotal role of the RPE-POS interaction for retinal health, further understanding of the underlying mechanisms of RPE plasticity in healthy and diseased retinas is an important research priority.

Establishing an iPSC-derived RPE disease model for RD-associated ciliopathy patients

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Purpose: Visual impairment affects at least 2.2 billion people worldwide. A large percentage can be attributed to genetic retinal diseases. Remarkably, more than 50 % of the genes responsible for retinal degeneration encode cilia-associated proteins. Expression defects of underlying genes can lead to ciliopathies such as Bardet-Biedl syndrome (BBS). Atypical *retinitis pigmentosa*, a rod-cone dystrophy, is one of the main manifestations of BBS. Most research on retinal degeneration has focused on the ciliated photoreceptor cells of the retina. However, recent studies have shown that the primary cilium in the retinal pigment epithelium (RPE) is essential for the development and functioning of the retina. This polarized, single-layered epithelium at the back of the eye is closely associated with photoreceptors and essential for retinal homeostasis. Human iPSC-derived RPE tissue is a promising tool for elucidating disease mechanisms and developing therapeutic approaches. Here, we differentiated both healthy and patient-derived iPSCs into RPE to identify possible pathogenic changes.

Methods: We differentiated human iPSC into RPE cells using a modified version of the Michelet *et al*. 2020 protocol. We analyzed healthy and *BBS1*^{mut} iPSC, RPE progenitors and fully matured RPE cells. We examined protein and gene expression, functionality (phagocytosis assay & TEER measurements), as well as morphology and ciliation.

Results: We were able to differentiate healthy and patient-derived iPSC into RPE cells. Furthermore, by analyzing iPSCs, RPE progenitor cells and mature RPE cells, we were able to detect early differences between healthy and *BBS1*^{mut} RPE. Matured RPE show potential differences in shape, tight junction formation and maturation rate between the healthy and the mutant cell line, which might have an impact on the functionality of the RPE.

Conclusion: In summary, we have established a disease model for *BBS1*^{mut} by differentiating patient derived RPE cells. This powerful tool allows us to examine the role of cilia patient mutations in the RPE and how this might contribute to retinal degeneration. By comparing different patient mutations, we hope to find common molecular mechanisms that can be targeted as possible therapeutic approaches.

In vivo analysis and long-term monitoring of cell transplants in the degenerative murine retina using retinal microscopy, fluorescence angiography and optical coherence tomography

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Purpose: Cell replacement therapies represent a promising approach for the treatment of retinal degenerative diseases that are characterized by the dysfunction and ultimatively loss of photoreceptors and/or retinal pigment epithelium (RPE). While functional assays such as electroretinogram measurements or behavioral tests can repeatedly be performed over the course of the experiment to assess retinal function, histological analysis is eventually performed several weeks after transplantation as an end-point analysis. Here, we assess and compare the potential of different non-invasive imaging techniques to monitor retinal cell transplantations *in vivo*.

Methods: Human iPSC-derived donor cells (RPE and photoreceptors) were subretinally transplanted into mouse models of retinal degeneration and a combination of retinal microscopy (RM, MicronIV), fluorescence angiography (FLA) and ultrahigh-resolution optical coherence tomography (OCT) was used to monitor changes in retinal structure longitudinally as well as to identify and trace donor cells within individual recipient mice weekly over the course of 14 weeks.

Results: We established an experimental pipeline that combines the three *in vivo* imaging techniques within the same imaging session repeatedly in the same animal. Obtained datasets could be combined with each other (e.g. re-identification of scanned retinal area using OCT in fundus images obtained using FLA) and showed alterations in the retinal structure (e.g. decreased thickness of retinal layers as indicator of retinal degeneration, loss of individual layers) as well as enabled the tracing of transplanted cells.

Conclusion: The combination of the three different *in vivo* imaging techniques allow a comprehensive insight into processes involved in retinal cell transplantations in experimental mice. These techniques are suitable for longitudinal monitoring of changes in retinal structure as well as identification of donor grafts. We are currently working on the optimization and automatization of our data analysis pipeline as well as the identification of suitable qualitative and quantitative parameters to assess, evaluate and potentially predict transplantation outcomes.

Recombinant human complement factor H CPV-101 and CPV-104 dampen microglia reactivity and attenuate light-induced retinal degeneration

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Purpose: AMD is a complex, heterogeneous and progressive chronic disease of the central retina that leads to severe vision loss among the elderly in the western world. Several lines of evidence including genome-wide association studies and preclinical model systems implicate microglia reactivity and dysregulated complement system in the pathogenesis of AMD. Genetic variants of complement factor H (CFH), an important regulator of the alternative pathway, are among the most prevalent polymorphisms that are associated with increased AMD risk. Here, we investigated whether moss-derived recombinant human CFH CPV-101 and CPV-104 have immunomodulatory and neuroprotective functions in a mouse model of light-induced retinal degeneration.

Methods: BALB/cJ mice received intravitreally injections of 5 µg CPV-101 and CPV-104 or vehicle, starting 1 day prior to exposure to 10,000 lux white light for 30 min. The effects of CPV-101 and CPV-104 treatment on microglia and Müller cell reactivity were analyzed by immunostainings of retinal sections and flat mounts. Gene expression of microglia markers was analyzed using quantitative real-time PCR (qRT-PCR). Optical coherence tomography (OCT); Blue Peak Autofluorescence (BAF); terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) stainings, and morphometric analyses were used to quantify the extent of retinal degeneration and photoreceptor apoptosis.

Results: Light-exposed mice treated with recombinant CFH CPV-101 or CPV-104 showed strongly decreased microglia and Müller cell reactivity concomitantly with reduced pro-inflammatory cytokines compared to vehicle controls. Furthermore, light-induced retinal degeneration was attenuated in mice that received CPV-101 or CPV-104.

Conclusion: Moss-derived recombinant human CFH CPV-101 and CPV-104 counter-regulates microgliosis and attenuates light-induced retinal degeneration, highlighting a promising concept for the treatment of AMD patients.

Microhomology mediated end joining based partial cDNA insertion into the *EYS* gene

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Purpose: Mutations in the *Eyes shut homolog* (*EYS*) gene are known to cause around 5 – 10 % of all autosomal recessive retinitis pigmentosa (RP25) cases. The EYS protein has a central role in the structural organisation of photoreceptors. Gene therapy of large genes is particularly challenging as adeno-associated viruses (AAVs) have only a limited cargo size of 4.5 kbp. A replacement of the mutation-containing 3'-prime part of the *EYS* gene by a wildtype cDNA repair template via Microhomology Mediated End Joining (MMEJ) DNA repair following a Cas9 induced double-stranded break (DSB) has potential to overcome the cargo issues of AAVs and therefore, offer treatment options for various disease-causing mutations within the *EYS* gene.

Methods: Four different Cas9 target sites, located in *EYS* exons 27 to 30, were characterised in an episomal BRET (Bioluminescence Resonance Energy Transfer) -based assay and verified using TIDE (Tracking of Indels by Decomposition) at the desired genomic locus. Varying micro homologous sequence (MHS) lengths (5, 10, 20 bp) were hybridised and cloned into wildtype cDNA repair templates containing the 3' exons 27 – 43, a polyadenylation signal and codon – optimised 5' – ends to prevent recleavage. The templates were purified for transfection into induced pluripotent stem cells (iPSCs) alongside corresponding Cas9/gRNA constructs. We then analysed MMEJ-based cDNA repair template integration using ICE (Inference of CRISPR editing) analysis and next-generation amplicon sequencing.

Results: All tested gRNAs showed promising integration efficiency during the plasmid-based BRET assay. On a genomic level, verified by TIDE analysis, the gRNAs targeting exons 27 and 28 showed efficiency rates of 9.7 % and 26.3 % respectively. The repair template was cloned into a vector containing a poly-adenylation signal. The native gRNA target sequence in exon 27 was replaced by a codon optimised MHS and for linearisation a SapI recognition site was added to the 5' end of the template.

Conclusions: The results presented in this study lay the groundwork for a MMEJ-based strategy aiming to integrate a larger *EYS* cDNA repair template to replace larger regions as we identified gRNAs which efficiently target 3' exons of EYS. The integration efficiencies remain low with the tested construct, yet the construct holds potential for an enhanced integration efficiency by creating a shorter MHS length.

A FRET-based biosensor for the analysis of opsin phosphorylation in health and disease

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Background: Rhodopsin and cone opsins are the the visual pigments in rod and cone photoreceptors, which absorb light and trigger a G protein-coupled receptor signal transduction cascade. A critical step in deactivation of the signal cascade and the recovery of the photoresponse is the phosphorylation of rhodopsin and cone opsins by the opsin kinases GRK1 and GRK7. Mutations in rhodopsin kinase (GRK1) and other proteins involved in the recovery phase of phototransduction are associated with congenital stationary night blindness. One prominent example is Oguchi disease, which can be classified based on two genotypes. Type 1 is linked to a deletion in the visual arrestin (SAG) gene, type 2 is caused by a mutation in GRK1. The aim of this project is to develop a versatile genetically encoded biosensor FRET (Förster resonance energy transfer)-based biosensor to investigate phosphorylation and dephosphorylation reactions.

Methods: The sensor consists of two fluorescent tags at opposite ends, mClover3 and mRuby3, which represent a FRET pair. A phosphoaminoacid binding domain (PAABD) is fused to the C-terminal end of mClover3 followed by the substrate region and mRuby3. When the substrate, rhodopsin or a cone opsin, is phosphorylated by the kinase of interest, it is recognized by the PAABD of the corresponding rod or cone arrestin. The complex formation between PAABD and phosphorylated substrate alters the orientation of the fluorescent proteins and causes detectable changes in FRET. The sensor is encoded by one single cDNA containing a cloning cassette which allows the exchange of compatible PAABD-substrate pairs. To study the functionality of the sensor, a proof of concept biosensor was designed with bovine rhodopsin as substrate for GRK1.

Results and conclusion: Functional expression of the sensor in HEK293 cells was verified by means of fluorescence emission spectroscopy and immunoassays. Fluorescence analyses showed a FRET signal of the sensor showing successful phosphorylation of the sensor by endogenous kinases (or GRKs) in cell culture. Our analysis indicated a heterogeneous phosphorylation pattern of the sensor, which will be further analysed by isoelectric focusing. Phosphatase treatment can reset the sensor to the non-modified state. The versatile design on the construct allows the investigation of different GRK isoforms and mutants on a variety of opsin substrates.

Generation of a human retinal organoid system to model the cholesterol storage disorder Niemann-PicktypeC

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Introduction: Niemann-Pick type C (NPC) disease is a cholesterol storage disorder caused by a malfunctioning NPC1 (95 % of patients) or NPC2 (5 % of patients) protein. NPC1 is located in the membrane of endo-lysosomal vesicles, which are part of a cell's transport machinery, responsible for shuttling cholesterol between the endoplasmic reticulum, the plasma membrane and the mitochondria. NPC1 dysfunction in patients leads to intracellular accumulation of cholesterol in lipid vesicles, e.g. liposomes, and mitochondria with brain and liver being the most affected tissues. This leads to severe symptoms similar to Alzheimer's and Parkinson's disease, as well as hepatosplenomegaly and visual impairments ultimately resulting in premature death by age of 25 years in early-onset patients. In a myeloid cell-specific NPC1 knockout (KO) mouse line, neurodegeneration was shown in brain tissue and neuroretina, highlighting the role of microglia in NPC (Dinkel et. al. in revision). Thus, we aim to mimic a retinal phenotype in NPC1-deficient human retinal organoids (hRO).

Methods: hRO were generated from both, a healthy human mother iPSC line as well as the NPC1 KO line derived from the latter. Typically, hRO do not contain microglia, because they are derived from the hematopoietic lineage, unlike hRO, which are differentiated from cells of the neuroepithelial lineage. Therefore, we co-cultured iPSC-derived microglia with hROs generated from the same iPSC line. By morphometric analysis of different cell populations using immunolabeling, the cellular composition of hRO was investigated.

Results: Both iPSC lines were shown to form hRO with the expected retinal layering. Quantitative analysis of calbindin-, calretinin-, recoverin- and vimentin-positive cells of cryosections from hRO of both genotypes at developmental day 70 (D70) showed no significant differences. Consistently, compared to the hRO derived from the mother iPSC line, VGLUT1-positive synaptic layers are similarly formed in NPC1 KO hROs from D100 onwards. This suggests that initial retinogenesis is not altered by NPC1 KO in this model. However, in NPC1 KO mice many cell types of the brain, including microglia, seem to be effected (1). Therefore, we suggest, that an effect of microglia in our hRO system can be expected. In co-culture experiments, microglia were shown to readily integrate into hROs regardless of the genotype and could be maintained in the organoids for up to 60 days.

Cholesterol accumulation in the microglial soma, a hallmark of NPC, was confirmed by filipin staining.

Conclusion and Outlook: Although initial retinogenesis and microglia integration appears to be unaffected by NPC1 KO hROs, there may be an effect on the maturation and/or survival of distinct cell populations at later developmental time points. Since cell number does not necessarily indicate cell functionality, we will characterize hRO glial cells in terms of their functionality upon NPC1 loss in live cell assays already established in the lab.

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Deletion of Versican V0/V2-isoforms affects retinal structure

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Purpose: Versican (VCAN), a chondroitin sulphate proteoglycan is a major component of the extracellular matrix. It consists of four isoforms (V0, V1, V2, V3) which differ in the respective glycosaminoglycan (GAG) attachment domain of the core protein. To learn more about the specific function of versican and its isoforms in the retina, we analyzed versican V0/V2-deficient mutant mice.

Methods: VCAN$^{(tm1Zim)}$ mice with a splice-variant specific gene inactivation of V0/V2 resulting in GAG-α domain deficiency were investigated. We analyzed the eyes of homozygous and heterozygous versican V0/V2-deficient mice in comparison to wildtype littermates during development on postnatal day (P) 1, 5, 15, and in adulthood. HE-stained paraffin- and semithin-sections were investigated by light microscopy. Investigation of the retinal pigmented epithelium (RPE), Bruch`s membrane and choriocapillaris was conducted via immunohistochemical stain against RPE65, ZO-1, and collagen VI. Retinal thicknesses were quantified in 4- and 8-week-old versican V0/V2-deficient and wildtype mice. The distribution of glial fibrillary acidic protein (GFAP) and the versican binding partner fibronectin was investigated by immunohistochemistry.

Results: The loss of V0/V2 isoforms resulted in formation of retinal rosettes, affecting the outer nuclear layer, and photoreceptor inner and outer segments and the RPE. Within the retinal rosettes pigment deposits and RPE cells could be identified. Retinal malformations ranging from mild to severe were first seen on P1 in mutant mice. Rosette formation was frequently associated with detachment of the sensory retina in adult mice. In contrast, Bruch`s membrane and choriocapillaris did not show obvious alterations. Moreover, retinal thickness was not altered in mutant mice. GFAP immunoreactivity in Müller cells of mutant mice was identical to that seen in wildtype littermates. Immunoreactivity for fibronectin was dramatically reduced in the retina. No alterations in RPE cell counts were detected between V0/V2-deficient and control mice.

Conclusion: Deficiency of the versican isoforms V0/V2 causes retinal changes that suggests its important role for the integrity of the sensory retina and the RPE.

Desialylation as a potential trigger of microglial responses in the aging and degenerating retina

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Age-related retinal degenerations are the major cause for vision loss and blindness. Hallmark of these diseases are chronically activated microglia and a pro-inflammatory environment in the retina. Major factors contributing to retinal immune dysbalance are reactive oxygen species (ROS) and complement components (C1q, C3) produced by microglia. Complement activation and microglial responses are controlled by surface sialylation and Siglec receptors as major inhibitory checkpoints. It is well known that the amount of sialylation is significantly reduced during aging, but the relevance for retinal degenerations is unclear.

This study investigates the effects of reduced sialylation on retinal microglia response and induced retinal degeneration in vivo. For this purpose, mice partially deficient in a key enzyme of sialic acid biosynthesis (*Gne*, UDP-*N*-acetylglucosamine-2-epimerase/*N*-acetylmannosamine kinase), combined with $Cx3cr1^{GFP/WT}$ microglia reporter animals are being used. $Gne^{+/-}$ x $Cx3cr1^{GFP/WT}$ mice and control animals are exposed to 10.000 Lux bright white light to induce retinal degeneration. Non-invasive spectral domain optical coherence tomography (SD-OCT) and blue-light fundus autofluorescence (BAF) are used to analyze retinal thickness and microglial response after light exposure, respectively. The sialylation state and microglia morphology is analyzed by fundus imaging and retinal flat mounts. qRT-PCR and RNA in situ hybridization are used to determine inflammation marker transcript expression and localization in the retina.

Our preliminary data showed that reduced sialylation was identified in the retina of 9-month-old $Gne^{+/-}$ mice. This correlated with an increase of microglia density as well as augmented gene transcription of pro-inflammatory factors. Further experiments are underway to study the effects of reduced sialylation in the light-damage paradigm of retinal degeneration.

Aptamer-based neuroprotection against oxidative stress in retinal organ cultures

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Introduction: Diseases such as glaucoma, retinitis pigmentosa (RP), age-related macular degeneration (AMD) and retinal ischemia lead to degenerative processes that result in vision loss and blindness. Apoptosis is the ultimate cause of photoreceptor and retinal pigment epithelial cell loss. Oxidative stress has a causal relationship to the pathogenesis of glaucoma, AMD and RP. The neurotrophic factor BDNF and its receptor TrκB are promising targets that support the survival of neurons and promote the growth and differentiation of new neurons and synapses. However, the use of BDNF as a therapeutic application is limited due to its short half-life and side effects. The aim of this project was to test the neuroprotective effect of an aptamer as a partial agonist of TrκB on porcine retinas damaged by oxidative stress.

Methods: Porcine retinal organ cultures were exposed to cobalt chloride ($CoCl_2$) or blue light (BL) to induce oxidative stress and apoptosis. Subsequently, retinas were treated with either the aptamer or BDNF as a comparison and cell viability, apoptosis and oxidative stress levels were measured. In addition, specific markers for retinal cells (Opsin, Rhodopsin, GFAP, β-III tubulin, PKCα), the TRKB signalling pathway (pAKT/AKT) and cell death were analysed at the mRNA and protein level.

Results: The TrkB-aptamer demonstrated effective activation of the TrkB receptor at a concentration of 100 nM, inducing neuroprotective effects similar to those of BDNF. Furthermore, with an extended treatment duration of 48 h, even a concentration as low as 10 nM led to notable receptor activation, as evidenced by the phosphorylation state of p-Akt. Treatment with the aptamer mitigated apoptosis induced by exposure to BL. Additionally, aptamer treatment successfully reversed retinal damage caused by $CoCl_2$ exposure.

Conclusions: The TrκB-aptamer activated specific and dose dependently TrκB downstream targets. A longer lasting effect of the TrκB-aptamer compared to BDNF treatment was observed. Overall, the aptamer had a neuroprotective effect on retinal ganglion, Müller and photoreceptor cells. This approach is therefore a promising approach for the development of therapies against various degenerative retinal diseases.

Prime Editing to correct a c5917InsAA frameshift mutation in the porcine *ABCA4* gene

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Purpose: Mutations in the human *ABCA4* gene are responsible for a diverse set of pathologies, chiefly among them Stargardt disease, an autosomal-recessive form of juvenile macular degeneration, representing the most frequent monogenic hereditary retinal disease and most frequent juvenile macular dystrophy. Causal therapies are not yet available, gene editing to correct the pathogenic mutations would however represent a potential candidate for such a therapy.
Prime Editing (PE) offers a therapeutic option to correct frameshifting mutations with high efficiency and low off target activity. A catalytically impaired nCas9 domain fused to a reverse transcriptase domain in combination with an elongated guide RNA (pegRNA) ("PE2") and optionally an additional nicking guide RNA (ngRNA) ("PE3") can correct these frameshifting mutations. BRET (Bioluminescence Resonance Energy Transfer)-based reporters offer versatile tools to assess editing efficiencies related on the correction of reading frames. The addressed frameshifting target mutation is introduced into the reporter's coding sequence which can be subsequently corrected restoring the original frame again, causing an altered emission pattern allowing the quantification of editing efficiency.

Methods: A double Adenine insertion (c5917InsAA) in Exon 43 of the porcine *ABCA4* gene was chosen as a model mutation to assess the editing efficiency. The target mutation was introduced into the *ABCA4* Exon43 containing BRET Reporter via mutagenesis PCR.
Peg- and ngRNAs were designed in silico, cloned, verified via sanger sequencing, and purified for transfection into HEK293T cells together with the BRET reporter system and Prime editors. After 72h of incubation, the cells were assessed via fluorescence microscopy and the BRET Assay.

Results: Sanger Sequence analysis confirmed success of peg-/ng-RNA design and cloning, as well as of the BRET-Reporter mutagenesis-PCR. Fluorescence Microscopy confirmed proper transfection. Fluorescence microscopy already showed a restoration of BRET-reporter GFP expression for both the PE2 and the PE3 system. The BRET assay used for the quantification displayed 2.8% ± 0.98% and 32.2% ± 8. 02% of editing for PE2 and PE3, respectively.

Conclusion: In HEK293T cells, Prime Editing was successful in correcting an AA insertion frameshift mutation with editing efficiencies of up to >30%. Additional PE3 nicking increases editing efficiency dramatically about 11.6-fold.
This study's results present a promising option for therapeutic intervention in Stargardt disease and potentially other hereditary diseases.

Targeting inosine-5'-monophosphate dehydrogenase 1 (IMPDH1) with mycophenolate as treatment for Retinitis Pigmentosa

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Purpose: Retinitis pigmentosa (RP) is a degenerative genetic disorder in which cell death can be connected to high cGMP levels in photoreceptors. This is exemplified by the *rd1* mouse model where a mutation in the *Pde6b* gene leads to decreased cGMP hydrolysis in photoreceptors. While guanylyl cyclase (GC) synthesizes cGMP in photoreceptors, the enzyme inosine-5'-monophosphate dehydrogenase-1 (IMPDH1) catalyses the rate-limiting step in the biosynthesis leading up to cGMP. Hence, inhibiting IMPDH1 may be a strategy for the reduction of photoreceptor cGMP levels and cell death. We explored the capacity of the registered immunosuppressive drug mycophenolate (MC) to reduce photoreceptor cGMP levels and cell death in *rd1* retinal explant cultures.

Methods: The retinal expression pattern of IMPDH1 and GC was assessed in wild-type and *rd1* mouse retina using immunofluorescence and Colocalization. Then, organotypic retinal explant cultures derived from post-natal day (P) 5 *rd1* mice were treated with MC in six different concentrations between 1 and 1000 µM. Parallel control cultures received vehicle or medium only. The retinal explants were cultured from P5 to P11 with medium changes every two days. At P11, the explants were fixed in paraformaldehyde, cryosectioned, and stained for cell death using the TUNEL-assay. TUNEL-positive cells were counted and compared with controls. To determine treatment effect on cones, these were quantified using Peanut agglutinin (PNA) labelling.

Results: IMPDH1 was expressed in photoreceptor inner segments, outer plexiform layer, neurites in the outer nuclear layer, and cell bodies in the inner nuclear layer. GC staining labelled the outer segments of photoreceptors. In *rd1 in vitro* explant cultures treated with MC, cell death decreased in a concentration-dependent manner. The concentrations 40, 78 and 250 µM significantly reduced cell death. However, at 1000 µM, cell death was strongly increased, and retinal structure was lost. No significant difference in cone cell numbers were found in the PNA staining.

Conclusion: The localization of IMPDH1 expression to photoreceptor inner segments makes it a potentially druggable target for the treatment of retinitis pigmentosa. Moreover, the treatment with MC did not seem to be toxic to retinal explant cultures but instead offers a potential neuroprotective effect.

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Prevention of photoreceptor loss by modulation of glucose metabolism

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Purpose: Mutations in the Crumbs homologue 1 (*CRB1*) gene can cause inherited retinal dystrophies such as Retinitis Pigmentosa (RP) and Leber congenital amaurosis (LCA). It has been established that *CRB1* patient derived retinal organoids have shown moderate disorganization of photoreceptor (PR) nuclei and dysregulated gene profiling of Müller glial cells and rod photoreceptors compared to their isogenic controls.
Here, we hypothesize that altering the glucose metabolism by ablating the prolyl hydroxyl domain 2 (*PHD2*) gene in *CRB1* patient human induced pluripotent stem cells (hiPSCs) could inhibit PR death and rescue aberrant phenotypes observed in the patient organoids by increasing the rate of aerobic glycolysis at the expense of oxidative phosphorylation (Warburg effect). This pathway provides PR with more glucose and allows phospholipid synthesis essential for the sustenance and renewal of the PR outer segments.

Methods: We generated a homology directed repair (HDR) mediated homozygous *PHD2* knockout (KO) in 3 independent *CRB1* patient iPSC lines by CRISPR-Cas9 gene editing. The guide RNA (gRNA) targeted exon 1 of *PHD2* with an aim to disrupt the region ahead of the catalytic domain responsible for hydroxylation and degradation of Hypoxia-inducible factor 1-α (*HIF1-α*), the master oxygen regulator. A single-stranded oligodeoxynucleotide (ssODN) sequence served as the template for the HDR, introducing a premature stop codon and an additional nucleotide to disrupt the open reading frame of *PHD2*, rendering the protein non-functional. The homozygote KO's were verified by sequencing and will be analyzed for copy number variations, karyotype stability and absence of off-target events.

Results: The exons coding for the catalytic domain of *PHD2* served as unfavorable targets for HDR mediated KO due to their limited size. Therefore, the gRNA targeting coding region of exon 1 was used to successfully obtain the KO iPS cell lines. Following further validation of the KO, the *CRB1PHD2* cell lines and their corresponding isogenic controls will be differentiated into retinal organoids.

Conclusions and future perspectives: The retinal organoids will be characterized by immunohistochemistry, scRNA sequencing, qPCR and metabolomics to analyze if the previously observed aberrant phenotypes in the PR are rescued. An improved PR structure is expected to be observed in these organoids due to the modified glucose metabolism.

Trained immunity in retinal degeneration

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Background: Age-related macular degeneration (AMD) and retinitis pigmentosa (RP) are the most common retinal degenerative diseases, leading to severe visual impairment. In retinal degenerative disorders, activated microglia and pro-inflammatory environments have been associated with the progression of the pathology. Therefore, the adaptive characteristics of innate immunity, or trained immunity, may induce the activation of resident microglia, predisposing to photoreceptor degeneration. While genetic predisposition plays a role, recent studies suggest that environmental factors have a critical contribution to the risk of AMD development. For example, obesity-related metabolic changes or systemic inflammation promote immune reprogramming, altering immune system function and response to subsequent stimuli. However, the contribution of trained immunity to the etiology of multifactorial and monogenic retinal degenerative disorders remains to be investigated. In this study, we explore the role of trained immunity in the development of maladaptive inflammatory responses in the retina using different mouse models.

Methods: BALB/c mice received different concentrations of fungal β-glucan (BG) or LPS intraperitoneally (ip) or intravitrealy (ivt) to study the metabolic reprogramming and the immune response of the retina. The retinal RNA was isolated 4 hours after injection and was analyzed further by qRT-PCR for expression of cytokines and enzymes involved in metabloic and epigenetic reprogramming. In the trained immunity model, BALB/c mice were first challenged with either 0.5 mg ip or 5 µg ivt BG. After 7 days, mice were subjected to 5000 lux white light for 20 minutes as second stimuli. Using qRT-PCR analysis, retinal gene expression was studied 4 hours after the light damage. Retinal degeneration and microgliosis were evaluated by optical coherence tomography and immunohistochemistry, respectively.

Results: Mice treated systemically or locally with LPS showed a stronger immune response in the retina than those treated with BG; however, only mice treated with an ip or ivt dose of 0.5 mg or 5 µg BG showed reprogramming of metabolic enzymes such as Suclg1, Fh-1, Mdh2, and epigenetic modifiers Set7 and Kdm5b. By applying these dosages of BG in our trained immunity mouse model, we observed more progressive retinal degeneration in mice primed systemically with BG but not locally upon light-induced damage. In consictancy, by immunohistochemistry analysis, a higher number of activated microglia were observed in the RPE and subretinal spaces of these mice. Gene expression analysis also revealed the upregulation of inflamatory markers such as Tnf-α in the retina of mice primed with systemic BG and subjected to light damage. Subsequently, sorted microglia will be used to determine chromatin modifications employing various sequencing techniques in order to further investigate the gene expression pattern and epigenetic basis of trained immunity.

Conclusions and perspectives: Our data suggest that BG preconditioning induces an immune memory in microglia and potentially myeloid cells that drives augmented inflammatory responses upon secondary stimulation in an AMD model. This finding will be further validated in other in vivo paradigms, such as the high-fat diet mouse model and the genetically induced RP model, Pde6b$^{rd10/J}$. Exploring the concept of trained immunity in the retina will have a significant impact on our understanding of the molecular mechanisms underlying detrimental chronic inflammation, not only in the context of retinal pathologies but also in neurodegenerative diseases in general.

Pharmacological targeting of inflammation in the pathogenesis of experimental diabetic retinopathy

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Background: Diabetic retinopathy (DR) is a vision-threatening complication of diabetes. Although the clinical manifestation of DR is associated with retinal microvasculature, neuroinflammation has been detected at the onset and progression of the human disease. Microglia, tissue resident macrophages of the retina, regulate inflammation and maintain retinal tissue homeostasis. However, sustained physiological stress can trigger chronic activation of microglia, which contributes to tissue damage. In order to identify and decipher the molecular and cellular causes of the disease, we mimic the vascular and neural complications of DR by inhibiting PDGFB/PDGFRβ signaling in mice.

Methods: C57BL/6J mouse pups received a single s.c dose of 30µg of anti-PDGFRβ mAb (clone APB5) at P1 to block recruitment of pericytes to endothelial cells. We generated the *Pdgfb*^{cECKO} mice by crossing Ve-Cadherin CreER^{T2} transgenic mice with PDGFB floxed mice (Pdgfb$^{flox/flox}$). For the treatment, mice received i.p injections of minocycline (45mg/kg) once daily from P5 – P9 or P27. The retinal vascular and neural changes at P10 and P28 were determined immunohistochemically and by in situ hybridization. RNAseq was used to analyse the retina transcriptome whereas qRT-PCR was used to validate differentially expressed genes. The thickness of the retina and vessel perfusion and fluorescein leakage were assessed by SD-OCT and fluorescein angiography, respectively.

Results: Inhibition of the PDGFB/PDGFRβ signaling pathway reproduced a phenotype reminiscent of human DR, characterized by both microvasculopathy and neuroinflammation. Inadequate pericyte cover and loss of PDGFB produced an altered vascular network with severe capillary deficits, microglia activation and impairment of retinal structure in young and mature retinae. Treatment with minocycline rescued capillary deficits, downregulated expression of inflammatory and angiogenic factors such as CCL2, iNOS, LGALS3, TSPO, VEGFA, PGF AND ICAM1 in young retinas. In the mature retinas treated with APB5 or the *Pdgfb*^{cECKO}, minocycline attenuated reactive gliosis, expression of EDN2, FGF2, CASP-1, LYZ2 and AIF-1 and preserved retinal structure.

Conclusion: Pharmacological modulation of inflammation by minocycline protects the retina from microvasculopathy and chronic inflammation in mice with an ocular phenotype reminiscent of human DR.

iPSC RPE cells help to understand and treat diverse retinal disorders

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Impairment and loss of vision are a leading cause of decreased quality of life. Patients with vision loss compared their burden to the burden of cancer. Diverse causes of visual impairment are different rare monogenetic diseases. However, the most prevalent form of visual deterioration is caused by age-related macular degeneration (AMD). AMD is a multifactorial disease affecting elderly people >55 years of age. Due to global demographic trends towards ageing population projections estimate 288 million affected people by 2040, posing a significant burden on quality of life and health care systems.

Most retinal diseases have limited or no available treatment option, leaving a high medical need for treatment. Although most of these retinal disorders have a different cause of illness, many share similar phenotypes and degenerative processes. Understanding these processes will help us to develop new therapeutic strategies.

In most retinal diseases retinal pigment epithelial (RPE) cells are dysfunctional prior to photoreceptor degeneration and vision loss. Therefore, we developed a screening platform using RPE cells derived from induced pluripotent stem cells (iPSCs). This gives us the opportunity to use cells from patients with diagnosed ocular diseases. For modeling purposes, we can produce large quantities of mature RPE cells and analyze their functional properties.

As a proof of concept, we used CLN3 knock out (ko) iPSCs, differentiated them towards RPE cells and compared these to wild type RPE cells. Here we show defects in the function of CLN3 ko RPEs. CLN3 ko RPEs show a decreased barrier function measured by transepithelial resistance, a deficient lysosomal activity and reduced phagocytosis of photoreceptor outer segments (POS).

For screening purposes, we developed a phagocytosis assay to evaluate phagocytic capacity of healthy RPE cells. Here we demonstrated a robust and screenable set up with stable and good assay windows, which enables us to perform assay development towards high throughput screening.

Our RPE screening platform enables us to investigate disease mechanisms and to find new molecular targets towards new treatment options for yet untreatable retinal diseases.

Vision restorative optogenetic gene therapy in presence of residual native vision? A mouse electroretinogram study

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Background/Purpose: Degenerative retinal disorders commonly lead to irreversible photoreceptor death. By rendering surviving inner retinal neurons light sensitive optogenetic gene therapy aims to restore lost vision, even after photoreceptor death, and widely independent of the underlying cause. While first clinical data from late-stage retinal degeneration patients is promising, tested approaches thus far have only managed to achieve restoration of very rudimentary vision, insufficient for performing complex visual tasks. One possible explanation therefore is that during degeneration the inner retina undergoes some degree of circuit rewiring that might hinder appropriate processing of the optogenetics-borne signals. Arguably, future optogenetic trials might need to be performed in patients with earlier stages of retinal degeneration before rewiring occurs and thus still with residual native visual function.
In order to test how residual native vision and optogenetics-borne signals would act together, we performed electroretinogram recordings from natively sighted mice expressing the optogenetic tool red-shifted channelrhodospin (ReaChR) in ON-bipolar cells.

Methods: Three groups of mice were included into the analysis: 1) $Grm6^{iCre/wt}$.ReaChR having normal native vision and additionally expressing the optogenetic construct ReaChR in ON-Bipolar cells (hereafter: “WT+ReaChR”). 2) Littermates, that were $Grm6^{wt/wt}$ and thus had native vision but not expressing the optogenetic construct (“WT-only”) and 3) mice that were $Grm6^{iCre/wt}$.ReaChR and additionally homozygous for the rd1 mutation in the Pde6b gene and thus without native vision at the time of experiment (“rd+ReaChR”). Electroretinogram (ERG) recordings were performed under isoflurane aesthesia at an age of 3 months using a Celeris ERG device (Diagnosys, Lowell, MA, USA) and flash stimulus intensities ranging from 0.001 to 150 (cd x s)/cm^2. Data analysis was performed using R and custom-made software (github.com/moritzlindner/ERGTools2).

Results: Optogenetically evoked light responses could be observed for stimulus intensities of 30 (cd x s)/cm^2 and above as a pre-a-wave negative deflection in the ERGs of “WT+ReaChR” mice. A similar waveform did not become apparent in the “WT-only” mice at any intensity and interestingly only from 100 (cd x s)/cm^2 onwards in “rd+ReaChR” mice. When compared to “WT-only” mice, in “WT+ReaChR” mice B-wave amplitudes were significantly reduced for light intensities up to 30 (cd x s)/cm^2, i.e. at intensities where no ReaChR would be activated. Piloting experiments recording visually evoked potentials indicate similarly lower signal amplitudes in “WT+ReaChR” mice on cortical level.

Conclusion: In the mouse model, native and optogenetically evoked light responses seem to interfere. In particular, native light responses get dampened while the optogenetic responses are enhanced. Thinking in terms of a possible optogenetic therapy in patients with residual native vision, such interaction would likely be unfavorable as the optogenetic vision is unlikely to outperform any residual native vision.

Optimizing the formulation of gene therapy for cone-rod dystrophies

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Background: Cone-Rod Dystrophy (CORD) affects around 1 in 30,000 people in the world causing severe vision loss. Autosomal dominant forms of CORD (adCORD) are associated with more than 20 mutations in the *GUCA1A* gene coding for GCAP1 (guanylate cyclase-activating protein 1), which is essential for maintaining photoreceptors viability.

Purpose: Gene therapy requires a limited number of administrations and could thus represent a viable option for the treatment of adCORD. However, DNA needs to be efficiently condensed for effective delivery to the retina. We present our preliminary results on the formulation of lipid nanovesicles loaded with DNA encoding for WT-GCAP1 exploiting (i) a biocompatible DNA-condensing agent to reduce DNA size and provide it with a positive charge, and (ii) a suitable drug delivery system that allows DNA to reach photoreceptors.

Methods: pCDNA3.1(+)-hGCAP1-mCar2 or pAAV-CMV-GFP plasmids – encoding, for a far-red fluorescent GCAP1-mCardinal2 fusion protein and green fluorescent protein (GFP), respectively – were firstly condensed with polyethyleneimine (PEI) for HEK293 cells transfection; protein expression was confirmed by confocal or fluorescence microscopy and Western blot analysis. Secondly, protamine (PRM) was used instead of PEI as a biocompatible condensing agent and its effectiveness in DNA condensation was monitored by Dynamic Light Scattering (DLS). Furthermore, the PRM/DNA complex was either encapsulated into liposomes or formulated as a solid lipid nanoparticle (SLN) and tested for its transfection efficacy and biocompatibility.

Results: Sequential additions of PRM to DNA was effective in reducing the hydrodynamic diameter. A minimum was reached at a PRM/DNA ratio of 0.05, resulting in a ~130 nm polyplex, which seems suitable for intraocular delivery. Both plasmids transfected HEK293 cells when condensed with PEI, whereas only pAAV vector shows this ability when condensed with PRM.

Conclusion: PRM is a biocompatible DNA-condensing agent which confers DNA the ability to transfect mammalian cells. The further formulation of this complex as liposomes/SLNs could improve its stability and mobility in the vitreous, thus making it a possible candidate for the treatment of adCORD.

Targeting choroidal endothelial cells: Capsid engineering of novel AAV variants for improved transduction

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Purpose: Age-related macular degeneration (AMD) is a common degenerative disease affecting the central retina (macula). A key feature is the formation of new choroidal blood vessels growing into and damaging the central retina. Implementation of anti-angiogenic drugs like aflibercept or brolucizumab has improved the prognosis of neovascular AMD. However, around 15% of patients do not respond to these treatments. And even if the treatments are effective against neovascularization, they cannot halt retinal degeneration. In addition, the treatments have to be repeated every one to two months, which is a considerable burden for patients. A potential curative solution might be gene therapy with adeno-associated virus (AAV) vectors targeting choroidal endothelial cells (EC) to counteract neovascularization. However, successful gene transfer to choroidal ECs remains challenging.

Methods: The engineered variants were based on AAV1, the AAV serotype with the highest reported efficiency in endothelial cells transduction *in vivo*. The engineered AAV1 variants carry the peptide insertions first described in AAV2.GL and AAV2.NN, previously identified AAV2 variants with enhanced transduction efficiency for retinal cells. First, we used human umbilical vein endothelial cells (HUVEC) as a screening platform to evaluate CMV-mediated GFP transgene expression of different engineered AAV capsids. Furthermore, we assessed these vectors on mouse primary choroidal endothelial cells to evaluate cell type specificity. Nevertheless, subretinal injections were performed to further confirmed the transduction efficiency of novel capsids in vivo. Finally, human choroid/RPE explants were cultivated to evaluate the selectivity and efficacy of novel AAV1-based vectors.

Results: The result indicated that modified AAV1 capsids transduce HUVEC with a higher efficiency. These novel vectors can also robustly transduce primary mouse choroidal ECs (transduction efficiency in the range of 60% and higher), in contrast to parental AAV1 or other naturally occurring serotypes. Efficiency of gene transfer in vivo in mouse choroid and ex vivo in human choroid/RPE explants showed promising transduction efficiencies in targeting primary choroidal ECs.

Conclusion: Our study identified new AAV1-based capsid variants can transduce choroidal EC efficiently, which can be confirmed by in vitro, in vivo, and human ex vivo culture. Our results showed that novel AAV1-based capsid could be applied for the gene therapy of AMD in the future.

Unveiling the potential of the long-term organotypic culture of the postmortem adult human retina

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Purpose: There is an increasing demand in research for three-dimensional human retina models. Here, we present an organotypic culture model that allows the postmortem adult human neural retina to survive in its full complexity for over 35 weeks.

Methods: Eyes of adult multi-organ donors without known ocular diseases were enucleated prior to cardiac arrest. Eyes from more than 150 donors, ranging in age from 18 to 74 years, were used in the study. After dissection, organotypic cultures were prepared and placed on a polycarbonate membrane. The cultures were maintained for up to 245 days using a chemically defined serum-free medium. Light sensitivity of the cultures and spontaneous ganglion cell activity were evaluated using a multi-electrode array system. To demonstrate a significant application of the method, a subset of cultures was transduced with different viral vectors. Following fixation, the quality of the cultures was analyzed by immunohistochemistry and TUNEL assay.

Results: The morphology of the cultures was remarkably well preserved, with low variability between samples. All major cell types survived, and the integrity of retinal layers was conserved even after 35 weeks. While cones exhibited a gradual loss of outer segments, they did not undergo severe apoptosis. In the inner retina, subpopulations of bipolar, horizontal, and amacrine cells showed morphology closely resembling the normal state. Additionally, synaptophysin staining unveiled synaptic structures with a similar normal-like morphology. Despite a reduction in the number of ganglion cells, the presence of surviving ganglion cells was confirmed by immunohistochemistry and electrophysiology. The applied viral vectors led to an effective and consistent transduction, demonstrating reproducibility across cultures from diverse donors.

Conclusion: Our results show that it is possible to maintain adult human retinas in a suitable culture system for at least 8 months. The long survival time and low inter-sample variability provide a cost- and time-efficient platform for evaluating pharmacological compounds on the human retina. In addition, long-term culture enables the delivery of viral vectors and opens up new and efficient strategies for the development and testing of gene therapeutics in the preclinical phase, which can significantly increase the success rate of clinical trials.

Role of exosomal miR-205-5p in angiogenesis and migration

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Purpose: Small extracellular vesicles (sEVs) represent a pivotal component in intercellular communication, carrying a diverse array of biomolecules. The dynamics of sEVs release are significantly influenced by the cellular microenvironment such as high glucose (HG). This phenomenon has been associated with the promotion of pathological processes. This study aims to understand the role of miR-205-5p in extracellular vesicles under hyperglycemic conditions and its effect on angiogenesis and cell migration.

Methods: ARPE-19 cells were cultured for 5 days with 35 mM glucose and then transfected with miR-205-5p mimic after 48 h cell culture medium was collected for sEVs isolation. The expression of miR-205-5p was detected with RT-qPCR. ARPE-19 and HUVEC cells were treated with sEVs to observe migration and angiogenesis processes. The sEVs were characterized using Nanosight, Western Blot, transmission electron microscopy (TEM), and stained with PKH26 for fluorescence internalization.

Results: After 35 mM glucose treatment for 5 days, ARPE-19 cells released a significantly higher number of sEVs ($3{,}74.10^8 \pm 1{,}30.10^8$ particles/ml). HUVEC cells treated with control sEVs exhibited lower uptake compared to cells treated with sEVs from 35 mM glucose conditions at 8 hours. Exposure to HG downregulated miR-205-5p expression in extracellular vesicles compared to control. Mimic transfection of this miRNA restores the values in HG conditions. HUVEC tube-formation was significantly increased by HG-sEVs from ARPE-19 (139.90 ± 7.09). This effect was attenuated by miR-205-5p mimic in sEVs 35 mM glucose+ mimic miR-205-5p (97.41 ± 6.70). In the wound healing assay, ARPE-19 cells with HG-induced sEVs (49.33 ± 15.88) showed faster wound closure compared to cells treated with control sEVs (31.75 ± 13.88), this effect was normalized by ectopic addition of miR-205-5p resulting in less migration in both conditions.

Conclusion: Our results demonstrate how ARPE-19 cells respond to high glucose challenges by increasing sEVs with low miR-205-5p content. The presence of this miRNA in sEVs can inhibit angiogenesis and migration opening new doors in cell-to-cell communication mediated by extracellular vesicles.

Comparison of different sorting procedures and analysis of cell proliferation in the context of RPE transplantations

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Purpose: Retinal pigment epithelium (RPE) degeneration induces photoreceptor dysfunction and loss and eventually leads to visual impairment and blindness. Cell replacement strategies using RPE cells derived *in vitro* from induced pluripotent stem cells (iPSCs) has emerged as a promising therapeutic strategy in recent years. Lately, we could show that human iPSC-derived RPE cells can form monolayer grafts covering substantial areas of the host mouse retina. Enrichment of a specific RPE subpopulation using cell surface markers led to further improvement of monolayer formation. Here, we analyze the influence of the cell sorting strategy on graft size and quality. Additionally, potential proliferation of transplanted RPE subpopulations within the graft was assessed.

Methods: RPE cells were differentiated from human iPSCs and sorted into specific subpopulations with the help of a previously established cell surface marker panel. Upon sorting using either the MACSQuant®Tyto® or the FACS ARIA™III, suspensions containing 50,000 target-enriched (TAR), target-reduced (RED) or unsorted control cells (CTRL) were transplanted subretinally into a mouse model of acute RPE depletion ($NaIO_3$ injection). Three weeks later, eyes were analyzed by immunohistochemistry. Animals transplanted with TAR, RED or CTRL cells (sorted using MACSQuant®Tyto®) as described above, received intraperitoneal injections of EdU (5 mg/kg BW) twice a day for four days prior to tissue collection to obtain an insight into proliferation dynamics within the graft.

Results: All transplanted cell fractions showed survival for three weeks and generated grafts with RPE-specific phenotypes irrespective of the applied sorting method. While retinal coverage and monolayer proporation appeared largely unaffected by the sorting method in TAR cells, we observed significant differences upon transplantation of the RED fraction (smaller grafts but higher monolayer proportion upon FACS). Furthermore, first results indicate the presence of EdU^+ cells in all grafts with the number of proliferative cells being higher in CTRL than RED and TAR donor cell subpopulations.

Conclusion: Here we demonstrate that the established marker panel for the enrichment of specific RPE subpopulations is applicable to different sorting methods. Nonetheless, sorting using the Tyto® seems to be superior over FAC-sorting as greater areas of the retina were covered by graft and this method could easily be adjusted to meet GMP-standards that would be required for clinical translations.
First results indicate that the level of cell proliferation *in vivo* within the graft is dependent on the transplanted RPE cell fraction. Further experiments will be required to fully understand and characterize the dynamics of cell proliferation in retinal cell transplantations.

Prph2 mutant mice reveal differential gene expression profile related to visual cycle forward degeneration

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Purpose: The Central Areolar Choroidal Dystrophy (CACD) is a retinal dystrophy caused by a single mutation in the Prph2 gene. This mutation, that affects photoreceptor outer segments, promotes a gradual breakdown of photoreceptors and retinal cells which ultimately results in vision loss. Using the CRISPR/Cas9 system, we developed a mouse model of CACD (Prph2 $^{KI/WT}$) by inserting the Arg195Leu mutation into the Prph2 gene. the same mutation that a Spanish family that suffers from this disease has. Our objective is to analyze the retinal mRNA changes of Prph2 $^{KI/WT}$ mice compared to control mice (Prph2$^{WT/WT}$), to understand the molecular mechanism involved in the degeneration process.

Methods: The neural retina from Prph2 $^{WT/WT}$ (control) and Prph2 $^{KI/WT}$ mice at different ages were used to perform transcriptomic analysis using RNA-seq total mRNA. We present the first differential expression analysis in Prph2 model between specified groups using R DESeq2 package. Genes with adjusted P value (< 0.05) and $\log_2$ fold change (≥ 1.0 or ≤ -1.0) were labelled as differentially expressed genes for each comparison. According to these results, enrichment analysis for biological processes and function pathways affected was performed. Moreover, we perform electroretinographic recordings (ERG) to evaluate the electrical response to light stimulus of retinas from the same mice.

Results: Our RNA-seq data shows that, among others, the visual cycle related genes such as RPE65, Rho and L-rat were already deregulated from first month of birth in Prph2 $^{KI/WT}$ compared to Prph2$^{WT/WT}$. This gene deregulation becomes more evident across the age when the electrical response of the retina is already greatly impaired. A decreased visual function was registered in Prph2 $^{KI/WT}$ compared to control mice in an age-dependent manner. However, the a- and b-wave values registered in ERG responses began to decrease after 6 months of age in Prph2 $^{KI/WT}$ compared to Prph2$^{WT/WT}$.

Conclusions: Related to happen in patients, the Prph2 $^{KI/WT}$ mice show a pattern of slow degeneration in their retinas. However, although the impairment of visual function in Prph2 $^{KI/WT}$ mice becomes evident from 6 six-month age, earlier alterations in the expression pattern of genes involved in visual cycle were detected.

Establishment of *MYO7A* knockout hiPSCs for generating Usher syndrome 1B human retinal organoids

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Purpose: Usher syndrome type 1B (USH1B) is caused by mutations in the *MYO7A* gene. Currently, there is no effective therapy for USH1B because of the lack of a faithful animal model and the limited knowledge of the pathomechanisms and the function of the MYO7A protein. Establishing USH1B human retinal organoid disease models can help us to better understand this disease as well as to test novel therapeutic approaches. For this purpose, we have disrupted the *MYO7A* gene in hiPSCs using the CRISPR-Cas9.

Methods: Two single guide RNAs targeting Exons 9 and 12 in the *MYO7A* gene were mixed with Cas9 nuclease protein to form a CRISPR-Cas Ribonucleoprotein complex that was delivered to a hiPSC line using 4D-Nucleofector®. Single cells were FACS sorted from the nucleofected hiPSC bulk cells. All single-cell colonies were analyzed at genomic and protein level. In addition, pluripotency markers were evaluated in the promising iPSC colonies using immunocytochemistry and qRT-PCR.

Results: The hiPSC bulk cells revealed a 2718 bp deletion between Exon 9 and 12 in the *MYO7A* gene. After the FACS sorting, forty single-cell colonies were developed and analyzed at the genomic level. Nine colonies showed the same deletion in both alleles. These nine colonies were further evaluated at the protein level using a western blot, and one colony showed a complete MYO7A protein loss. The pluripotency markers were expressed in all promising colonies. Whole genome sequencing is in progress to analyze off-target effects and to further confirm the MYO7A knockout.

Conclusions: We identified one hiPSC colony with a complete MYO7A loss at the protein level. This colony will be further used to generate the MYO7A KO human retinal organoids as a model for USH1B.

Dietary treatment of mice for antioxidant lipid substitution using deuterated polyunsaturated fatty acids (D-PUFAs)

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Purpose: One hallmark of age-related macular degeneration (AMD) is the deposition of metabolic byproducts behind the retinal pigment epithelium (RPE) and in the Bruch's membrane. These so-called 'drusen' increase in size and number, compromise the RPE cells and lead to a loss of photoreceptors, impairing the patient's visual field. We focus on the susceptibility of polyunsaturated fatty acids (PUFAs) to oxidative stress, specifically linoleic (LA), arachidonic (AA) and docosahexaenoic acid (DHA) which are located in the lipid membranes of photoreceptors. Replacing protium with deuterium in these molecules, due to the kinetic isotope effects, significantly increases their resistance to oxidative stress. We aim to investigate if deuterated fatty acids, administered by diet, are incorporated into the membranes of the cells in the posterior retina, also looking for specific distribution patterns and any potential negative effects on retinal structure and functionality.

Methods: Male and female adult mice (C57BL/6J) are given a specific diet, supplemented with the modified fatty acids. Other animals are provided with the non-deuterated version. Additional mice are maintained as a control group with conventional nutrition. At specific stages, the following parameters are assessed: weight, structural characteristics of retinal layers, retinal perfusion and function. Following completion of the protocol, the eyes are extracted postmortem for further analysis, in particular by mass spectrometry.

Results: So far, no alterations in retinal morphology have been observed following the feeding of deuterated or non-deuterated PUFAs. Both groups showed a slight reduction in electroretinographic amplitudes, without significant differences between them. Evaluation of the cryosections revealed that D10-DHA is predominantly incorporated into the outer segments of the photoreceptors. D2-LA is elongated to D2-AA and is present in the inner and outer nuclear layers as well as in the RPE.

Conclusions: As of now, more animals need to be included in the study to achieve statistical reliability. Experiments with arachidonic acid have just begun and are so far consistent with the results of the other two fatty acids. Demonstrating that D-PUFAs have no harmful side effects prompts subsequent research to determine whether they also contribute to enhanced resistance of the retina to oxidative stress.

The retinal degeneration stage influences incorporation of donor photoreceptors

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Purpose: Retinal degenerative diseases culminate in loss of photoreceptors, the light-sensing cells of the retina, leading to vision impairment. Cell replacement has been proposed as a therapeutical strategy to restore vision, e.g. by transplanting iPSC-derived photoreceptors into the degenerated retina. While structural incorporation of human photoreceptors has been shown, little emphasis has been placed on the microenvironment of the host and how it may influence this process. Here, we assess how the extent of outer nuclear layer (ONL) degeneration influences survival, distribution, and incorporation of donor photoreceptors after transplantation.

Methods: Towards this purpose, the transgenic mouse model Cpfl1/Rho$^{-/-}$ (tgCR), presenting progressive ONL degeneration, was employed. Following the *in vivo* and *in vitro* characterization of this model, distinct levels of degeneration were allocated to an early (<90%), midterm (50%), and late (>90%) ONL degeneration stage. Using an established protocol, the CRX-mCherry line - a photoreceptor reporter hiPSC line - was differentiated into retinal organoids. Post sorting at day 200 *in vitro*, a suspension of 150.000 mCherry^{+} cells was transplanted subretinally into wild-type and tgCR mice at the defined degeneration stages. Ten weeks post transplantation, the eyes were enucleated and processed further for immunohistological analysis.

Results: Early (<90%), midterm (50%), and late (>90%) ONL degeneration stages correponded to 4, 7 and 16 weeks old tgCR mice, respectively. Assessment of the experimental retinae revealed donor cell survival at ten weeks post transplantation in all degenerated stages. While donor photoreceptors mainly appeared in cell clusters and showed signs of structural incorporation in early degeneration stage hosts, in the later degeneration stages donor cells appeared mostly isolated and were distributed over a larger part of the retinal surface. Notably, donor cells showed signs of maturation (i.e. hMito^{+} protrusions resembling inner segments) and interaction with Müller Glia processes more prominently in the early than in the late stage transplanted retina.

Conclusion: First results indicate that a residual endogenous ONL might provide an advantageous niche for subsequent incorporation of donor cells within the host retina. Müller glia appear to play a major role, forming the first contacts with donor cells, and therefore could drive the process of incorporation. Further evaluation of host-donor interactions is crucial for delineating factors that potentially influence transplantion and could render cell replacement therapy successful.

The role of miRNAs in dormant mouse cone photoreceptors and human retinal organoids

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Purpose: Photoreceptor-specific (miR-182/183) and neuronal (miR-124) miRNAs are important for cone photoreceptor maintenance, survival and function. We asked whether overexpression of these miRNAs protects cone outer segments (OS) from degeneration in mouse models of retinitis pigmentosa (RP) and promotes cone OS formation in human stem cell-derived retinal organoids (hROs).

Methods: To overexpress these miRNAs, their precursor sequences were placed within an intronic sequence followed by a GFP reporter gene. This genetic cassette was driven by a cone-specific promoter and transduced by AAV-mediated gene transfer into photoreceptors of 1-day-old retinal degeneration mice 1 (rd1) and rd10. Vision-guided behavioral tests were performed 6, 9, and 13 weeks after injection. To monitor successful transduction of cone photoreceptors, we analyzed the GFP signal *ex vivo*. Retinas were also isolated for *ex vivo* electrophysiological recordings and subsequent immunohistochemistry. We transduced 19-week-old hROs with increasing concentrations of AAV. Age-matched untransduced hROs served as controls. The hROs were fixed three weeks post-transduction (pt) and processed for vibratome sections. Cone specific antibodies were used to follow the outgrowth of cone OS.

Results: Subretinal injection of AAV resulted in robust expression of GFP in the photoreceptors of retinal explants in *rd1* mice. However, a statistically significant preservation of visual acuity in *rd1* mice could not be confirmed. *Ex vivo* recordings showed no light-coupled activity. For the *rd10* model, vision-guided behavioral testing, *ex vivo* retinal recording and immunohistochemistry are ongoing. At the highest concentrations used for both AAVs, hROs showed GFP signals in cone photoreceptors 5 days after pt. HROs transduced with lower concentrations showed GFP signals 1 week after pt and increased over time. Immunohistochemical and miRNA expression analyses are ongoing.

Conclusion: To date, we could not confirm whether miRNAs have a protective effect in the *rd1* mouse model, which is known for early onset and rapid retinal degeneration. The analysis of their protective nature in the *rd10* model and their potential to enhance cone maturation in hROs will help to exploit their full potential on cone photoreceptor OS in health and disease.

Innate immune response in atrophic AMD: Inflammasome and microglia activation by lipofuscin-mediated photooxidative damage

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Introduction: Age-related macular degeneration (AMD) is the leading cause of blindness in all industrialized countries. The disease is characterized by accumulation of insoluble material at the level of the retinal pigment epithelium (RPE) in the form of both intracellular lipofuscin and extracellular drusen. Atrophic AMD is characterized by well-demarcated atrophic lesions resulting from loss of RPE cells followed by degeneration of photoreceptor cells, which results in vision impairment. Chronic local inflammation including inflammasome and microglia activation plays a central role in AMD pathogenesis.

Purpose: This study aims to investigate the role of NLRP3 inflammasome activation in a mouse model mimicking aspects of AMD, i.e. light-induced retinal degeneration (LIRD) in ABCA4/RDH8 double (*Abca4$^{-/-}$/Rdh8$^{-/-}$)* and ABCA4/RDH8/NLRP3 triple knockout mice (*Abca4$^{-/-}$/Rdh8$^{-/-}$/Nlrp3$^{-/-}$)* that accumulate excessive lipofuscin in the RPE.

Methods: We plan to induce LIRD by white light exposure. Subsequently, markers of retinal inflammasome activation will be analyzed, such as mRNA and protein levels of IL-1β, IL-18, NLRP3, GSDMD, ASC-1 and caspase-1. Cell death will be assessed by TUNEL staining, and retinal microglia activation and subretinal migration by immunohistochemical Iba1 staining. We expect increased light damage and inflammasome activation in *Abca4$^{-/-}$/Rdh8$^{-/-}$* mice compared to *Abca4$^{-/-}$/Rdh8$^{-/-}$/Nlrp3$^{-/-}$* mice and wildtype controls.

Results: In previous *in vitro* experiments, white light exposure of lipofuscin-loaded murin and human RPE cells resulted in pronounced NLRP3 inflammasome activation with release of IL-1β and IL-18 as well as secondary cell death by pyroptosis. Apically secreted cytokines induce microglia activation and migration. Selective NLRP3 inhibitors are capable to suppress these processes *in vitro* and thus show promise as therapeutic strategy in atrophic AMD. In vivo analysis for *Abca4$^{-/-}$/Rdh8$^{-/-}$* mice show increased light damage associated loss of retinal thickness compared to wildtype controls. Further analysis is in progress.

Inhibition of high glucose-induced Retinal Angiogenesis by miR-205-5p: Targeting HIF-1α and VEGFA expression

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Purpose: Diabetic Retinopathy (DR), one of the leading causes of blindness in adults worldwide, is characterized by critical complications such as retinal angiogenesis and endothelial cell dysfunction. Emerging evidence suggests that miR-205-5p acts as an anti-angiogenic agent across several organs. This study aims to explore the hypothesis that miR-205-5p mitigates retinal damage in hyperglycemic conditions by modulating the expression of Hypoxia-Inducible Factor 1-alpha (HIF-1α) and Vascular Endothelial Growth Factor A (VEGFA), thereby inhibiting angiogenesis.

Methods: Angiogenesis assays were conducted using HUVEC cells exposed to conditioned medium from ARPE-19 cells treated with 35 mM glucose, transfected with miR-205-5p mimic, and/or treated with 4 mM N-Acetylcysteine (NAC). The total tube length from images was quantified by Image J using the Angiogenesis Analyzer plugin. The expression levels of VEGFA and HIF-1α mRNA in ARPE-19 cells under different experimental conditions were quantified using quantitative real-time PCR (qRT-PCR), with relative expression analyzed through the 2-ΔΔCt method. Statistical differences among groups were determined using one-way ANOVA followed by Tukey's multiple comparison test, presenting values as mean±SEM (angiogenesis assay, n=3; qRT-PCR, n=4).

Results: Enhanced tube formation in HUVEC was observed with the addition of medium from ARPE-19 cells treated with 35 mM glucose (202.4±28.7). This angiogenic effect was significantly attenuated by the conditioned medium from ARPE-19 cells treated with NAC (127.9±12.24), miR-205-5p mimic (111.7±12.57), or their combination (113.1±15.53). Overexpression of miR-205-5p led to a decrease in HIF-1α mRNA expression under both control conditions (0.372±0.097) and HG conditions (0.341±0.037). Furthermore, it decreased VEGFA mRNA expression exclusively in HG conditions (0.605±0.103). Additionally, the addition of NAC to the miR-205-5p mimic did not produce additional effects, proposing an absence of synergistic interaction between the two.

Conclusions: Overall, our findings demonstrate that miR-205-5p plays a pivotal role in inhibiting retinal angiogenesis by downregulating HIF-1α and VEGFA expression. These results suggest that the upregulation of miR-205-5p could represent a novel antiangiogenic therapeutic strategy for DR.

Identification of RPE specific WNT inhibitors to improve RPE maturation and function

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Purpose: The retinal pigment epithelium (RPE) is essential for visual function and is often affected in retinal degeneration (RD) disease mechanisms. iPSC-derived RPE is currently being developed as a treatment for ocular diseases including ciliopathy associated retinal degeneration. We previously found that the primary cilium regulates RPE development via modulation of WNT signalling, which can be manipulated to improve iPSC-RPE maturation and function. Therefore, identifying WNT inhibitors that expedite and improve this differentiation process is an important aspect of translational research.

Methods: To find RPE specific WNT inhibitors we investigated the effect of a selection of known and novel WNT inhibitors on RPE cell lines. We treated three RPE cell lines namely ARPE19, RPE-J and hTERT RPE-1 with three known and two novel WNT inhibitors. We performed Western blot and RT-qPCR to analyse their effect on gene and protein expression, and immunofluorescence microscopy to examine cell morphology.

Results: The tested substances showed an effect upon the canonical WNT signalling pathway. Some WNT targets were less expressed, however not all tested substances had the same effect. Intriguingly, in addition to the known inhibitors, treatment with one of the novel substances also lead to a downregulation in protein and RNA expression of WNT target genes.

Conclusion: Known WNT inhibitors and newly found substances were shown to have an effect on various different RPE cell lines. This makes them possible candidates for improving iPSC-RPE maturation and function. In future we will apply the most effective RPE specific WNT inhibitors to ciliopathy-patient-derived iPSC-RPE to determine whether we can rescue the disease phenotype.

Characterization of a mouse model of complex outer retinal pathology by injection of HBEGF and TNF for photoreceptor transplantation

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Purpose: Retinal degenerative diseases, like age-related macular degeneration, present a complex retinal pathology, characterized by photoreceptor loss and glial pathology. However, its recapitulation is limited in preclinical models. Recently, Völkner et al. (2022) showed that the combined application of Heparin-binding EGF-like growth factor (HBEGF) and Tumor necrosis factor-α (TNF-α ; H+T) induces complex retinal pathology in human iPSC-derived retinal organoids. Here, we assess whether these factors induce a complex retinal pathology also in the adult mouse eye, and its effect on photoreceptor transplantation.

Methods: C57BL/6j mice at 16 weeks of age received intravitreal injections of HBEGF and TNF-α separately or combined at different concentrations for 2, 3, or 4 days. Eyes were analyzed post treatment by TUNEL assay and IHC using antibodies against photoreceptors and Müller glia. Additionally, H+T-injected mice received a subretinal transplantation of day 200 photoreceptors, enriched from human iPSC-derived retinal organoids (Crx-mCherry iPSCs line). Retinas were analyzed 3 weeks later.

Results: Major retinal foldings, cell death in the outer nuclear layer (ONL), ectopic cells, GFAP up-regulation and SOX2 migration was observed after 4 days of H+T injections, with the pathologic changes persisting for at least 30 days. Photoreceptor degeneration was present after 2 days of H+T injection at standard concentration and 3 days of H+T diluted 1:10. Upon transplantation of human photoreceptors, increased Müller glia-graft interactions were observed in H+T in comparison to sham-injected mice.

Conclusion: Overall, H+T injections rapidly induce a complex retinal pathology phenotype in adult mice, with the phenotype depending on injection number and concentration. This model provides an *in vivo* complex retinal pathology which might help in the study of retinal degeneration mechanisms. Furthermore, the H+T model appears to facilitate host Müller glia-donor photoreceptor interactions, relevant for the understanding of photoreceptor transplant integration and the role of Müller glia for structural graft incorporation into the diseased retina.

1. Vöilker M, Wagner F, Steinheuer LM, Carido M, Kurth T, Yazbeck A, Schor J, et al. HBEGF-TNF induce a complex outer retinal pathology with photoreceptor cell extrusion in human organoids. Nature communications. 2022. Acknowledgments to the ERA-Neuro-Net consortium ReDiMoAMD. BMBF/ERANET Neuron ReDiMoAMD (grant number: 01EW2106)

Rational design and in vitro screening of novel AAV variants for improved retinal microglia transduction

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Purpose: Recombinant adeno-associated virus (AAV) vectors have been widely used in gene therapy applications for central nervous system (CNS) diseases and have yielded favorable preclinical and clinical outcomes. These vectors can successfully transduce post-mitotic cells, such as neurons and astrocytes. Emerging evidence indicates that the microglia, the major resident immune cells in the CNS, play a central role in the pathobiology of neurodegenerative disorders. Consequently, these cells have recently become the focus of new treatment approaches. However, these approaches have been hindered by the difficulty in transducing microglia using viral and non-viral vectors.

Methods: To address this, here we introduce a novel microglia culture protocol from mouse retina, which is reproducible and is characterized by high cell numbers and long *in vitro* viability. We used this model as a screening platform to evaluate CMV-mediated reporter transgene expression of engineered AAV6- or AAV1-derived capsids, the most used serotypes on glial cells. The variants carried single-point mutations of surface-exposed tyrosine, lysine, threonine, serine residues, and/or arginine residues, or the peptide insertions of AAV2.GL and AAV2.NN, two previously described 12-mer peptide insertions that enhance retinal cell transduction when introduced into the AAV2 capsid.

Results: The variants carrying 12-mer peptide insertions, AAV-GL and AAV-GL.R, showed enhanced transduction efficiency of primary mouse retinal microglia cells. With the best-performing AAV1 variants, we could reach more than a 10-fold increase over AAV1 wild-type and around 2,5-fold compared to the recently published triple mutant AAV6 TM6. This improved activity has been confirmed in both resting and chemically activated microglia and allowed us to achieve the first (epi)genome editing in the targeted cells. Finally, we started to validate our variants in mouse models of retinal disorders. In Rd1 mouse models with abundant microglia, we were able to reach 20% of Cd11b+Cd45+ cells after intravitreal injection of AAV1 variants.

Conclusions: Overall, our study establishes a new *in vitro* platform for assessing microglial transduction and identifying novel AAV capsids with improved microglial cell transduction properties. The in vitro selected variants show enhanced transduction efficiency of microglia also in vivo. Future studies will focus on combining the novel capsids with microglia-specific promoters for restricted and cell-specific targeting.

Investigating the regenerative and proliferative competencies of human retinal pigment epithelial cells

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Retinal degeneration is amongst the leading causes for impaired vision and complete blindness. Some pathologies involve the depletion of retinal pigment epithelium (RPE) during aging. Contrary to fetal RPE cells, which possess proliferative capacities, adult RPE cells that are lost due to injury or disease fail to regenerate and replenish the damaged structures leading to photoreceptor degeneration and vision loss. Current therapies are successful in slowing disease progression but fail to restore vision. Thus, there is a need to develop strategies to counteract RPE and photoreceptor loss.

To this end, we aim to identify factors that can induce RPE replenishment by utilizing two systems. First, we focus on the regenerative capacity of the Xenopus laevis retina and utilize an in vitro assay for RPE dedifferentiation and retinal regeneration. By applying scRNAseq to RPE cells undergoing stages of migration, proliferation and dedifferentiation, we are identifying key candidate factors for RPE replenishment.

Second, we developed a novel in vitro injury model to assess the proliferative capacity of hESC-derived RPE of different ages. While, young RPE cultures can proliferate upon cell loss, old cultures show decreased proliferative capacities, leading to hypertrophy of the remaining RPE cells and contributing to RPE dysfunction as seen during aging in-vivo. Comparisons of the transcriptomes led to the identification of potential pro-proliferative candidates. In the future, we will integrate the information gained from these two systems to test candidate factors that can restore cell division in adult RPE cells. This will lead to novel strategies to restore eyesight in non-regenerative mammalian species.

Automated quantification of photoreceptor outer segments in developing and degenerating retinas on microscopy images across scales

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Purpose: The functionality of photoreceptors is highly dependent on outer segments (OS). These membranous structures generate biochemical signals from incident light. While OS formation and degeneration is qualitatively assessed on microscopic images, reliable methodology for quantitative analyses is still limited. Here, we developed quantification methods to evaluate OS maturation and quality using automated image analysis.

Methods: OS formation was examined during development and in adulthood (between 4 days and 35 weeks of age) of C57Bl/6JRj wild-type mice via light and transmission electron microscopy (TEM).
To quantify number, size, shape and fluorescence intensity of OS, retinal cryo-sections were immuno-stained for s-opsin. Fluorescence images were used to train a classifier based on supervised machine learning for automated image segmentation. Characteristic features of predicted labels were extracted to quantify maturation of cone OSs. Subsequently, this quantification method was applied to characterize OS degeneration in 'cone photoreceptor function loss 1' (Cpfl1) mice between 8 days and 35 weeks of age.
TEM images were used to establish an ultrastructural quantification measurement for alignment of OS membrane stacks. Images were analyzed using a custom written MATLAB code to extract the orientation of membranes from the image gradient and their alignment (coherency). We used this analysis to quantify the OS morphology of wild-type and two inherited retinal degeneration ('retinal degeneration 19' (rd19) and 'rhodopsin knock-out' (RhoKO)) mouse lines at 1 month of age.

Results: Both automated analysis technologies provided robust characterization and quantification of OS based on fluorescence microscopy or TEM images. Automated assessments showed an increase in OS number, volume, and membrane coherency during wild-type postnatal development, while a decrease in all three observables was detected in different retinal degeneration mouse models.

Conclusion: Automated image segmentation by a classifier based on machine learning and analysis of the orientation of membrane stacks using fluorescent or TEM images, respectively, allow quantitative evaluation of photoreceptor OS formation and quality. These approaches are useful for in depth analysis of OS in developmental studies, for disease modelling or after therapeutic interventions affecting photoreceptors.

Inflammatory protein profiling in aqueous humour of patients with non-exudative age-related Macular Degeneration

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Purpose: A dysregulated immune system, particularly the complement system, plays a fundamental role in the pathophysiology of age-related macular degeneration (AMD). The aim of this study was to analyse complement components and inflammatory mediators in the aqueous humour of non-exudative AMD patients and to compare them with a control group.

Methods: In this study, 12 non-exudative AMD patients and 21 controls were enrolled. Aqueous humour was collected during cataract surgery and 78 complement components and inflammatory proteins were measured using multiplex immunoassays. The influence of sex or smoking on the AMD status was assessed. Biomarker levels between AMD patients vs. controls, smokers vs. non-smokers and females vs. males were compared. Moreover, associations between drusen volume and biomarker levels as well as subject age and biomarker levels were examined.

Results: All investigated immunological factors were detected in the aqueous humour. The proteins were categorised into high, medium and low-level concentration groups. Aqueous humour contained abundant complement proteins, including C3b/iC3b, FH/FHL-1, C4 and FI. Lower levels of C4B (P = .020), IL-10 (P = .032), FI (P = .082) and higher levels of SDF-1α (P = .085) were observed in non-exudative AMD patients. Drusen volume positively correlated with CCL4 levels (r_s = .78, P = .013). Additionally, smokers showed significantly higher levels of pro-inflammatory proteins (CCL7, IL-6, IL-7; P = .027, P = 0.28, P = 0.30). MMP-1 was positively correlated with age (r_s = .43, P = .013). Sex differences were observed in FB (P = .027) and C4 (P = .036).

Conclusion: This study presents the broad spectrum of inflammation-associated biomarkers measured in aqueous humour and indicates possible roles of C4B and IL-10 in non-exudative AMD development. Further biomarker investigations locally at the eye site are important for a better understanding of AMD and potential treatments.

Microhomology mediated end joining based partial cDNA insertion into the *USH2A* gene

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Purpose: Mutations in the *USH2A* gene are one of the major causes of autosomal recessive Retinitis Pigmentosa (arRP). These mutations can occur in a non-syndromic or syndromic form, as a combination of visual and hearing impairment. Treatment of various *USH2A* mutations remains a challenge as there are no causal therapies. However, one promising approach is the use of the DNA repair pathway microhomology mediated end joining (MMEJ). This strategy uses Cas9 to create a targeted double-strand break in the mutated *USH2A* gene, allowing repair mechanisms to replace the defective region with a wild-type cDNA template. This offers the hope of targeting different disease-causing mutations and potentially improving treatment outcomes.

Methods: A Cas9/gRNA target site was characterised for exon 60 in the *USH2A* gene. Wild-type cDNA repair templates were designed, which contained different micro-homologous sequences (5, 10 and 20bp). In addition, the 5'-end codon was codon-optimised to prevent re-cleavage after successful integration. For transfection into induced pluripotent stem cells (iPSC), together with a Cas9/gRNA construct, the repair templates were cloned and purified. The online tool ICE-Analysis (Inference of CRISPR Edits) and Next Generation Sequencing will be used to analyse the successful MMEJ-induced cDNA repair template integration.

Results: A backbone PCR was successfully performed to integrate the various MHS oligonucleotides into the vector using blunt-end ligation. The vector was successfully cloned and the repair templates were cut out by restriction digestion. Successful transfection of the iPSC was demonstrated using a GFP control. The MMEJ integration rate is currently under investigation using ICE-Analysis and Next Generation Amplicon Sequencing.

Conclusion: This study's findings provide the foundation for an MMEJ-based strategy to replace mutated regions by integrating a larger *USH2A* cDNA repair template. By comparing the different lengths of MHS, we were able to optimise error-free cDNA template integration as a potential therapeutic option for *USH2A*-associated diseases.

Effects of 85 % hyperoxia after 14 days postnatally on retinal angiogenesis in iNOS-KO and wild type mice

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Purpose: The aim of this study was to examine the impact of postnatal exposure to 85% hyperoxia in comparison to normoxia (21%) on retinal vascular development in mice. Furthermore, the study will shed light on possible protective effects associated with inducible nitric oxide synthase (iNOS) deficiency.

Methods: To visualize the complete retinal vascular network, eyes were dissected, the retina separated and flattened. The different vascular plexus were analyzed in frozen retinal cross-sections. For analysis of blood vessels immunohistochemical (IHC) staining with Isolectin, GFAP, Iba1, αSMA and Coll TypIV were applied .

Results: Isolectin Ib4, which stains endothelial cells, showed a similar outcome in both wildtype and iNOS-knockout mice at postnatal day 14. All animals held in normoxia exhibited physiological and extensive retinal vascularization. The superficial plexus, which develops first, was already fully formed and contained vessels with a well visible lumen. The deep plexus, known not to begin vascular growth until p7, was not fully matured. The last developing, intermediate plexus, showed only initial stages of its final appearance. A different picture emerged when looking at both mouse lines kept in hyperoxia. None of the three plexus began to develop in the hyperoxic environment and no vascular network was observed. Individual vascular bundles were identified that seemed to be overlying the retinas of animals in 85% oxygen. All animals kept in normoxia exhibited a physiological and distinct GFAP immunopositive astrocyte-cell-network that served as a template for the developing vessels. In hyperoxia, astrocytes were also present, but more isolated and did not show a well-developed network.
Iba1 immunopositive microglia cells showed initial evidence of differences between iNOS-KO and wild-type mice in their total number, their shape, and presumably their activity.

Conclusions: In hyperoxia, all mice showed inhibited development of the cellular network of astrocytes and the blood vessels in the retina. First findings suggest persistent hyaloid vasculature to compensate for the missing plexuses. In iNOS-deficient mice, no differences or protective effects regarding retinal angiogenesis were observed yet. However, in microglia IHC first evidence of differences between the mouse lines were detected.

ARMS2 A69S polymorphism enhances retinal degeneration in a human RPE-porcine retina co-culture model for AMD

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Purpose: Age-related Macular degeneration (AMD) is a degenerative disease of the macula affecting the elderly population, resulting in photoreceptor (PR) cell death and subsequent vision loss. A specific genetic variant, identified as a missense single nucleotide polymorphism in the *ARMS2* gene (rs10490924) in Chr. 10q26 locus, p.A69S, is a frequently observed AMD risk allele in the human population. We have previously determined ARMS2 expression in human retinal pigment epithelium (RPE) cells. Little is known about the impact of this variant and in particular how this variant affects the neuroretina. This study investigates the effects of the *ARMS2* AMD high-risk A69S variant in RPE cells on the retina in a hybrid co-culture model composed of human RPE cells and porcine retinal explants.

Methods: hTERT-RPE1 (RPE) cells were seeded on transwell inserts and transfected with *ARMS2* non-risk and A69S variants, and an empty expression vector. Overexpression of constructs in RPE cells was validated by Western blotting via ARMS2 antibody. After 24h, the transfected RPE cells were exposed to porcine retinal explants (from the visual streak of the porcine retina, which is rich in cone PRs) for co-culture experiments. Following a 72-hour incubation period, co-cultures were fixed and sectioned for imaging (mean±SEM, n=6). PR cell survival was determined by counting the DAPI-stained cell rows and measuring the percentage of TUNEL-positive cells in the outer nuclear layer (ONL). Alterations in cone PRs and the neuroinflammatory response were monitored by immunofluorescence staining for opsin and microglia, respectively, with cell-specific antibodies.

Results: Retinae co-cultured with *ARMS2* A69S RPE cells showed enhanced retinal degeneration compared to *ARMS2* non-risk RPE. Specifically, we observed a significant increase in the number of dying PR cells in the ONL when retinae exposed to *ARMS2* A69S RPE (measured as %TUNEL: 4.02 ± 0.62%) compared to *ARMS2* non-risk RPE (%2,5 ± 0,42). Moreover, retinae co-cultured with *ARMS2* high-risk RPE cells displayed a lower count of PR cell rows in the ONL (6.19 ± 0.2) compared to those co-cultured with *ARMS2* non-risk RPE (7.02 ± 0.19). Notably, those retinae also exhibited a diminished number of cones (number of cones in 100 µm: 28 ± 0,43 vs 10,54 ± 0,74) and displayed more microglia activation (Iba-1 positive cells in the ONL: 2,3 ± 0,29 vs 1,18 ± 0,17) when co-cultured with *ARMS2* A69S RPE compared to *ARMS2* non-risk RPE.

Conclusions: Our data show that RPE cells overexpressing the *ARMS2* AMD high-risk variant contribute to enhanced retinal degeneration in the porcine neuroretina compared to *ARMS2* non-risk cells. The results strongly imply a connection between the *ARMS2* A69S polymorphism in RPE cells and loss of PR as seen in AMD patients. We believe that these results are essential for advancing our understanding of AMD and have the potential to contribute to the development of therapeutic interventions.

Vascularization of human stem cell-derived 3D retinal organoids

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Purpose: Retinal organoids (ROs) are 3D *in vitro* structures that attempt to recapitulate the development, structure, and functioning of *in vivo* human retina. RO field has evolved over time meticulously regulating features of organoid protocol like structural complexity, scalability and reproducibility. However, inefficient exchange of oxygen and nutrients and periodic cellular waste removal generates an expanding necrotic core causing aggressive ganglion cell (GC) death resulting in premature functional decline. Vasculature within the developing organoid has shown to combat above mentioned drawbacks. Here, we make use of an efficient hiPSC-derived inducible endothelial cell (EC) differentiation protocol that gives rise to an endogenous, self-organizing vascular-like system within the retinal layers prolonging the survival and long-term functioning of RO.

Methods: 3D-ROs were generated using human iPSC line F49B7 using (Agarose Microwell Array Seeding and Scraping) AMASS protocol. To incorporate vasculature, B7 line was transduced with a construct containing endothelial lineage driving transcription factor ETS variant 2 isoform 2 (ETV2.2) and GFP under TetOn system. Upon merge of B7-derived embryoid bodies and pre-differentiated B7-derived ECs, a network of growing vasculature was successfully located within retinal layers. Impact of vasculature was analyzed using various downstream assays. Data was normalized to non-vascularized controls across three independent batches.

Results: The vascular-like network was visualized using EC markers such as collagen IV and CD31. Stainings revealed presence of network architecture branching out into thinner capillaries innervating the outer and inner nuclear layer of vascularized ROs (vROs). Vascular lumens were stained with EC markers and a tight junction marker a-ZO1. This data was also corroborated with qRT-PCR across the vRO development. vROs were significantly bigger in size which was a result of increased rate of oxygen diffusion that alleviated cell apoptosis in mature vRO. Also, these vRO for the first time show higher survival of GC indicating preserved function. Functional output upon vasculature is currently under investigation.

Conclusions: An optimized protocol for vascularization of ROs generates a complex and branched capillary mesh innervating retinal layers. Presence of lumens assist in efficient diffusion of nutrients and oxygen alleviating necrosis, recuperating growth, and even preserving function in older ROs. Advanced vROs are key to exploring retina physiology and function in biomedical research.

Establishing plasmid-mediated expression of RNA-based TrκB-binding aptamers for the treatment of age-related macular degeneration

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Purpose: Apoptosis is the predominant mechanism responsible for the loss of photoreceptor and retinal pigment epithelium (RPE) cells in age-related macular degeneration (AMD) – the major cause of blindness in people over 50. Interestingly, the anti-apoptotic brain-derived neurotropic factor (BDNF) levels in the aqueous humour of patients suffering from AMD are significantly decreased compared to healthy volunteers. However, treatment with BDNF leads to overstimulation of the TrκB receptor and severe side effects were observed. To overcome this hurdle, a partial agonistic RNA-based TrκB-binding aptamer (TrκB-aptamer) is used to compensate for the decreased BDNF levels. The neuroprotective effect of a singular aptamer administration to the *ex vivo* retina is presented in the poster of Hurst *et al.* To ensure a constant supply of the aptamer and to overcome the ongoing degradation of the RNA, the goal of this EU-funded project is to continuously express TrκB-aptamer using the portable plasmid-mediated ToRNAdo expression system.

Methods: The TrκB-aptamer was cloned into the ToRNAdo expression system. In this system, the TrκB-aptamer is flanked by ligation sequences and autocatalytic ribozymes. After transcription from the U6 promoter by RNA polymerase III, the ribozymes cut and induce 5'OH and 2'3'cyclic phosphate on each side of the aptamer, and the ligation sequences hybridize. The endogenous RtcB enzyme recognizes the 5'- and 3'-end and ligates them together to form the circularized TrκB-aptamer.
hTERT-RPE1 and primary porcine Müller cells (ppMüller cells) were transfected with the cloned plasmid using Viafect and Fugene combined with CombiMag, respectively. The transfection efficiency was determined using microscopy and flow cytometry. The *in vitro* toxicity was evaluated with an MTS assay.

Results: After cloning, the presence of the TrκB-aptamer in the expression system was confirmed by Sanger sequencing of the plasmid. hTERT-RPE1 and ppMüller cells were successfully transfected with the ToRNAdo expression system containing the TrκB-aptamer – reaching a transfection efficiency of up to 88.13% in hTERT-RPE1. Furthermore, non-toxic *in vitro* expression was established in hTERT-RPE1 cells and ppMüller cells since there was no significant difference in relative cell viability between transfected and non-transfected cells.

Conclusion: The TrκB-aptamer was cloned into the ToRNAdo expression system to ensure continuous expression. Both hTERT-RPE1 and ppMüller cells were transfected, and non-toxic expression of the TrκB-aptamer-containing ToRNAdo expression system was established in hTERT-RPE1 and ppMüller cells.

Rescuing the deep intronic *USH2A* variant c.7595-2144A>G by AAV-packageable enhanced-deletion RNA-guided endonucleases

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Purpose: Usher syndrome is the most common form of hereditary deaf-blindness. Various genes are causative for the condition, one of the most frequently affected being *USH2A*. A recurrent deep intronic variant (*USH2A* c.7595-2144A>G) is the underlying genetic cause in up to 4% of Usher patients. This variant causes an mRNA splicing defect resulting in aberrant or truncated protein. By using novel engineered enhanced-deletion synthetic RNA-guided nucleases (EDsRGNs), this study aims to develop a safe therapeutic strategy to target the described pathogenic variant. These chimeric proteins of the RNA-guided endonuclease sRGN3.1 and the human exonuclease TREX2 generate enhanced deletions at the target sites whilst using only a single gRNA. While ensuring significant perturbation of the disease-causing sequence, the nuclease engineering aims to incorporate important safety features.

Methods: Two newly engineered EDsRGN nucleases coupled with six different gRNAs were tested for their splicing rescue potential harnessing minigene splicing assays. Therefore, HEK293T were co-transfected with an *USH2A* c.7595-2144A>G-minigene and an EDsRGN-gRNA-encoding plasmid, followed by transcript analysis. Additionally, these rescue experiments were performed by delivering the nuclease-gRNA combination by recombinant adeno-associated viruses (rAAVs). Occurrence of adverse chromosomal translocations upon gene-editing treatment was examined by interchromosomal junction PCR. Next generation sequencing (NGS) was used to analyze editing efficiency and deletion profiles.

Results: In minigene splicing rescue assays, up to 90% of correct *USH2A* transcript was detected when transfecting EDsRGN nucleases and a gRNA directly targeting the variant. Delivery of the editing machinery via rAAV transduction led to equal and substantial splicing rescue efficacy for all variants. PCRs detecting hybrid chromosomes showed nearly undetectable occurrence of chromosomal translocations with the EDsRGN variants as opposed to sRGN3.1, while NGS proved comparable editing rates. Genomic deletion profiles clearly demonstrated that EDsRGN variants induce enhanced and directional deletions.

Conclusion: With our novel recombinant endonucleases, significant rescue of aberrant *USH2A* mRNA splicing could be achieved *in-vitro.* EDsRGNs induce enhanced deletions and offer safety features compared to alternative CRISPR-Cas methods. Moreover, these engineered nucleases can be efficiently delivered by all-in-one rAAVs. Ongoing experiments include unbiased assessment of potential off-target effects and protein-rescue experiments in AAV-transduced retinal organoids harboring a 3xFLAG-epitope tag fused to the C-terminus of endogenous *USH2A*, aiding protein detection.

RNA base editing in iPSC-derived retinal cell models to correct a common CRB1 mutation

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Retinitis pigmentosa (RP) is an inherited retinal dystrophy that leads to visual impairness and blindness. Many of the causative genes for RP are responsible for photoreceptor or retinal epithelial cell functionality such as phototransduction, the visual cycle or homeostasis. Amongst them, is the Crumbs complex protein CRB1, with over 150 identified mutations causing missense changes or null alleles. The most common mutation in the *CRB1* gene is C948Y, caused by a single base pair exchange from guanine to adenine at position 2,843 of the longest *CRB1* splice variant. Although several gene therapeutic approaches are being explored, there is no treatment option for retinopathies to this day. Furthermore, in case of CRB1, a variety of isoforms with different length and expression pattern complicate their development.

Although classical gene therapy methods may enable the modification or replacement of the mutated *CRB1* gene, the associated risks of the treatment are too high. A potential alternative to classical gene therapies involves the use of a novel class of oligonucleotides (ASO) which harness endogenous A-to-I RNA editing enzymes to correct G-to-A base pair exchanges as for instance the C948Y mutation in *CRB1*.

Using a modified approach called RESTORE (recruiting endogenous ADAR to specific transcripts for oligonucleotide-mediated RNA editing), a restoration of the disease-causing point mutation C948Y in *CRB1* was achieved. As an initial *in vitro* model for the treatment, we used hiPSC-derived Müller glia cells, considering them as a potential target cell type for the therapy. Using transfection and electroporation of *CRB1*-targeting ASO, we achieved 10-40 % genotypical point mutation correction.

As a next step, we explored the potential of ASOs to penetrate retinal tissue by using retinal organoids derived from RP affected patients and from healthy donors. To find the optimal transduction technique for 3D tissues, a variety of methods such as lipofection, electroporation, magnetofection and nanoparticles is explored. The correction efficiency of the point mutation in the 3D retinal organoids was lower compared to 2D, highlighting the importance of three dimensional cell models. This sets the ground for the development of further RESTORE ASOs to target inherited point mutations with unmet clinical needs.

Understanding the molecular mechanisms and modifiers underlying *CRB1*-associated retinal degenerations

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Purpose: *Crumbs homologue 1 (CRB1)* is one of the major genes associated with autosomal recessive Retinitis Pigmentosa (RP). More than 450 variants have been described in CRB1 patients, most of which are located in the extracellular domain of the transmembrane protein. While the protein interactions of the intracellular domain have identified CRB1 as a key regulator of cell polarity, the functions of the extracellular domain are poorly understood. In addition, modifiers of disease severity are insufficiently investigated. Consequently, no treatment is available to prevent photoreceptor degeneration in these patients.

Methods and Results: To assess the molecular mechanisms and modifiers underlying CRB1-associated retinal degenerations, two siblings, which were diagnosed with RP and carrying a homozygous CRB1 c.2843G>A;p.C948Y mutation were investigated. Interestingly, the two patients displayed a substantial difference in the disease severity. Using western blot analysis and immunofluorescence microscopy, we show that total CRB1 protein levels are significantly reduced in iPSC-derived retinal organoids (RO) from both patients compared to the control, suggesting that the CRB1 levels do not correlate with the disease severity. To identify potential modifiers of disease severity, the retinal interactome of CRB1 was investigated using a porcine retinal pull-down approach and mass spectrometry analysis. In addition, whole genome sequencing and whole proteome analysis of patient and control RO were performed. These data revealed protective and harmful candidate modifiers, that may protect the less affected patient from a severe phenotype or accelerate the symptoms in the other patient, respectively. One of the protective candidates identified was CRB2. Using co-immunoprecipitation, we show that human CRB1 and CRB2 interact homotypically and heterotypically at the OLM. Furthermore, human CRB2 was able to pull-down endogenous CRB1 from porcine retinal tissue suggesting a strong interaction in the retina. In addition, we observed a high overlap between the retinal interactome of CRB1 and CRB2, suggesting that CRB1 and CRB2 may regulate similar cellular processes.

Conclusion: Conclusively, we provide novel potential modifiers in two siblings with RP carrying the homozygous CRB1c.2843G>A;p.C948Y mutation, which serve as a proof-of-concept to further validate modifiers in CRB1-linked retinal degenerations.

Retinal organoids to study retinal degenerative processes: Oxidative stress assays in a 3D model

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Age related macular degeneration (AMD) is a major cause of irreversible blindness in the western world. The current treatment options for patients with AMD are very limited and most clinical trials fail due to lack of efficacy. The poor translatability of in vitro findings to the human situation is caused by oversimplistic cell models, which fail to capture the complexity of the human retina. Oxidative stress is associated with AMD and other retinal diseases, such as diabetic retinopathy and retinitis pigmentosa. When oxidative stress exceeds the endogenous capacity of cells to return to homeostasis it becomes pathogenic, contributing to the retinal degeneration. The development of screening and profiling assays of disease relevant features, such as oxidative stress, in retinal organoids offers a real opportunity to identify new therapeutical targets for AMD and other forms of retinal degeneration. Here, we developed retinal organoids as a tool to screen for new targets and antioxidant drugs that could provide neuroprotection to retinal cells. We show that Menadione can be used to induce oxidative stress, which can be directly detected by live imaging in 96well format, enabling mid throughput drug screening campaigns, as well as a novel tool for compound profiling. The induction of oxidative stress in retinal organoids is further confirmed by histology and single nuclei RNA sequencing. Here, we see a dose dependent response, with photoreceptor and Müller glia cells showing the greatest stress response and upregulation of several relevant transcriptomic pathways. Pre-treatment with antioxidants led to a reduced induction of oxidative stress response. This is the first *in vitro* oxidative stress assay, in human 3D models, which lends itself for drug screening approaches. Furthermore, our data suggest that retinal organoids are a potent tool to study oxidative stress, identify targets and screen for compounds that could impact AMD development and progression.

Functional evaluation of *CNGB1* variants associated with Retinitis Pigmentosa for their potential to affect mRNA splicing

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Purpose: Retinitis Pigmentosa (RP; rod-cone dystrophy) is a rare, genetically heterogeneous inherited retinal disease with a prevalence of ~1/5,000 worldwide. It is characterized by rod degeneration followed by secondary cone photoreceptor loss. *CNGB1* encodes the modulatory B1 subunit of the rod photoreceptor cyclic nucleotide-gated ion channel, an essential component of the phototransduction cascade. Variants in *CNGB1* – associated with autosomal recessive RP – account for <5% of RP cases, and several variants have been described that are located at or in proximity to exon-intron junctions but do not affect the canonical splice sites essential for mRNA splicing. Such variants are often of uncertain clinical significance, although prediction softwares (i.e. Splice AI, MaxEntScan, TraP score) propose an effect on splicing. To understand their impact on *CNGB1* splicing and to elucidate their disease-relevance, *in vitro* minigene splice assays were conducted.

Methods: Wild-type and mutant multi- but also single-exon minigenes were created by PCR amplification and *in vitro* mutagenesis. These minigenes were transfected individually into HEK293T cells. After RNA isolation and cDNA synthesis, PCR amplified splicing products were analyzed by visualization on agarose gels and direct Sanger-sequencing. If needed, splicing products were further analyzed by sequencing of subcloned fragments and/or fragment analysis.

Results: Data mining of in-house and public mutation databases (i.e. ClinVar, LOVD) as well as literature revealed 26 *CNGB1* candidate variants. A first set of eight variants was functionally analyzed. Minigene splice assays showed no effect on splicing for three variants. For the remaining five variants, exon skipping, intron retention and the use of cryptic splice sites could be confirmed. Six of eight variants studied were initially classified as variants of uncertain significance (VUS) following the ACMG/AMP guidelines. Using these minigene data, five VUS were re-classified as (likely) pathogenic, and 1 as likely benign. One variant was confirmed as benign. No splicing defect was observed in the likely pathogenic variant affecting the second nucleotide of *CNGB1* exon 30, indicating that this variant likely acts as a missense rather than a splice variant.

Conclusion: The use of *in vitro* minigene splice assays is an effective tool to assess the pathogenicity of putative splice variants.

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Peroxisome proliferator-activated receptors promote photoreceptor survival in the *rd1* mouse model for retinitis pigmentosa

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Purpose: Peroxisome proliferator-activated receptors (PPARs) function as transcription factors that regulate the expression of genes linked to various physiological and pathologic processes, including to energy metabolism. In some ocular diseases, PPARs may play a role in inflammation and angiogenesis. However, their impact on retinitis pigmentosa (RP) remains unclear. This study tested the hypothesis that PPAR regulation can rescue photoreceptors in the *rd1* mouse model.

Methods: Organotypic retinal explants were derived from *rd1* mice and cultured from post-natal day 5 to 11. Retinal explants were treated from post-natal day 7 onwards with agonists for PPARα, PPARβ/δ, and PPARγ, respectively, to evaluate their impact on photoreceptor survival. After histological workup, cell death in the retina was examined using the TUNEL assay. The activities of poly-ADP-ribose-polymerase (PARP) and calpain-type proteases were assessed using specific assays. One-way analysis of variance (ANOVA) testing was used for statistical analysis.

Results: PPARα and PPARγ agonist treatment significantly reduced the numbers of TUNEL-positive cells in the *rd1* outer nuclear layer (ONL), when compared with untreated *rd1* retina. Likewise, PARP activity and accumulation of its product poly-ADP-ribose (PAR), also displayed a significant reduction in treated *rd1* ONL, compared to untreated. However, PPARβ/δ agonists did not reduce ONL cell death. Further photoreceptor degeneration markers, namely calpain activity and calpain-2 activation, were elevated in untreated *rd1* retina. PPARγ agonists significantly reduced both overall calpain activity and calpain-2 activation, while PPARα or PPARβ/δ agonists had no significant impact on calpain activity.

Conclusions: Our results indicate that PPAR activation may be beneficial for *rd1* photoreceptors since activation of PPARα and PPARγ increased photoreceptor viability. Notably, PPARγ may rescue photoreceptors by regulating both PARP and calpain activity. This study contributes to our understanding of the degenerative mechanisms governing RP and may provide new targets for therapeutic intervention. Future research should explore the efficacy of PPAR agonists across different RP models and their associated metabolic or signaling pathways.

Keywords: PPAR, PARP, calpain, cell death mechanism, energy metabolism

AAV transduction of porcine retinal explants

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Purpose: The porcine retina represents an optimal model system to study inherited retinal dystrophies due to the close anatomical similarities to the human retina, including a cone enriched *area centralis*. As large animal models require significant resources and are therefore often difficult to perform, the organotypic retinal culture represents a valuable alternative model system to study AAV-mediated gene therapeutic approaches prior to *in vivo* application. In this project, we tested several capsid-engineered AAVs on porcine retinal explants and analyzed their transduction efficiency compared to *in vivo* data.

Methods: Retinal explants were cultured on semipermeable polycarbonate membrane inserts with the photoreceptors facing down. 20 µl of the different GFP-encoding AAVs (AAV2, AAV2-7m8, AAV2.GL, AAV2.NN) containing $5x10^{10}$ vector genomes were used on top of the ganglion cell side. After 7 days in culture, explants were harvested, processed for cryosections and subsequently stained for different cell-specific markers to characterize GFP-positive cells and their location.

Results: After 7 days in culture, the explants showed GFP-positive photoreceptor and Müller glia cells after transduction with all tested AAVs. The number of transduced cells, however, increased with the engineered capsid variants in comparison to the wildtype AAV2.

Conclusions: Using a standardized protocol, porcine retinal explants represent an easy to handle intermediate model between *in vitro* and *in vivo* experimentation. Here we could prove that capsid-engineered AAVs for intravitreal use have a comparable transduction efficiency in porcine retinal explants than they do in other model systems and *in vivo*. Therefore, we can conclude that testing novel strategies in a porcine retinal explant model prior to performing *in vivo* studies can provide valuable insights and can contribute to lowering animal numbers.

USH1C retinal organoids provide novel insights into Müller glia and photoreceptor pathology of human Usher syndrome and offer promising treatment options

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Purpose: Usher syndrome (USH) is the most common cause of hereditary combined deaf-blindness in human. To date, there is no treatment to slow down or prevent blindness. Reasons for this are the lack of reliable animal models that show an ocular phenotype and are suitable to unravel pathomechanisms of retinal degeneration. Thus, we focus on the generation of *USH1C*$^{R31*/R80Pfs*69}$ retinal organoids (ROs) as a cellular disease model. We aim to decipher *USH1C*/harmonin's role as a scaffold protein in RO Müller glia cells (MGCs) and photoreceptor cells (PRCs) to gain insight into the molecular pathology. Furthermore, we established a screening platform for the identification of translational readthrough inducing drugs (TRIDs) to favor overreading of nonsense mutations.

Methods: We reprogrammed healthy and *USH1C* patient-derived fibroblasts into induced pluripotent stem cells (iPSCs) and differentiated them into mature ROs. Single cell RNA-sequencing (scRNA-seq) of 19,173 cells from healthy and *USH1C* ROs was conducted and Uniform Manifold Approximation and Projection (UMAP) was assessed. We performed Differential Expressed Gene (DEG), Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis focusing on MGCs and PRCs. ROs were morphologically characterized and the localization of marker proteins was determined by immunofluorescence analysis. For the identification of novel TRIDs as a therapeutic option, we stably transfected s-harm-a1-R31*-nLuc HEK293T cells and screened 528 fungal extracts via a luciferase assay. Cell viability was measured in a WST-1 assay.

Results: ROs show characteristic layering. scRNA-seq revealed 29 cell clusters in healthy and *USH1C* ROs, corresponding to the majority of cell types in the human retina. We identified a 19% increase of MGCs and a 25% decrease of rod PRCs in *USH1C* ROs. GO-term analysis of significant DEGs in MGCs revealed dysregulation in various biological processes and cellular components, e.g. *Wnt signaling* and *extracellular vesicles*. In rod PRCs we found DEGs enriched for *detection of visible light* and *photoreceptor outer segment*. Moreover, KEGG analysis of rod and cone PRCs showed significant downregulation of the *phototransduction cascade*. Accompanying with this, a decreased brush border length and outer limiting membrane (OLM) thickness was determined in mature *USH1C* ROs. In our TRID screening, we identified one promising extract having a better readthrough activity than the clinically approved drug Translarna.

Conclusion: Overall, our studies offer promising therapeutic options through fungi-based TRIDs and demonstrate the utility of ROs to unravel the molecular pathology of USH, shedding light on cellular dysregulation in MGCs and PRCs.

Key ethical competencies for resolving power and knowledge imbalances in participatory health research. Results of a participatory workshop.

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Purpose: Participation in research is increasingly demanded by the scientific community, third-party funders, and patient organisations (PO). Despite this demand, knowledge and power imbalances as well as diverging interests and expectations exist between stakeholders, promoting value conflicts. On the one hand, a PO may hope that the scientists involved in the research project will also communicate their findings to their members. On the other hand, the scientific team is primarily interested in publishing their findings for the international research community. In our presentation we want to show the potential for conflict that lies in these different interests and expectations and to what extent this can be aggravated by an imbalance of power. Fundamentally, we will point out how the power and knowledge imbalances can be recognized, communicated and overcome.

Methods: In our presentation, we will use two exemplary case studies to outline what specifically lies behind power and knowledge imbalances and how miscommunication as a decisive factor can lead to pseudo-participation in research projects. The case studies were prepared in a co-produced workshop with members from PO and scientists. The aim of the joint workshop was not only to derive alternative actions for participation in research, but also to identify the necessary competences.

Results: The results show an urgent need for POs to receive structural support at the institutional level to professionalise for scientific collaboration. The results also illustrate that conflict potential can be reduced by acquiring ethical competencies. Here reflection on one's role in the collaboration as well as openness to the perspective of other stakeholders are central. In addition, the goals, interests and expectations of all stakeholders must be communicated and discussed transparently from the outset (expectation management) and equal cooperation should be ensured by a cooperation agreement.

Conclusion: Transparent communication and openness to the perspectives of others takes time. Therefore, we argue that participatory research projects must receive a longer funding period to ensure successful cooperation at eye level.

The economic impact of inherited retinal diseases: A systematic literature review.

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Background: Retinal disease is one of the leading causes of blindness and vision impairment worldwide. Particularly, inherited retinal disease (IRD) is of concern as it often results in significant or total vision loss in young patients. Furthermore, the recent market entry of Luxturna® to treat Leber congenital amaurosis (LCA) presents gene therapy as a promising option for IRD patients in the future. As such, given the rarity of IRD and the high price of innovative medicines, a question ensues as to the feasibility and equity of caring for these conditions. It is thus essential to consider the current costs associated with IRD and their potential implication in terms of drug costs in the future.

Aim and objectives: We aim to investigate the economic impact of inherited retinal disease.

Methods: Following a dedicated PICOS framework, we systematically searched the Medline, CINAHL, EconLit and Embase databases to identify economic studies estimating costs (direct as well as indirect) associated with IRD in patients. The searches were restricted to English language articles published between January 2000 and February 2024. Data synthesis as well as quality assessment will be conducted according to the PRISMA guidelines. The protocol of this study (ID: CRD42024501626) is registered in the international database of prospectively registered systematic reviews, PROSPERO.

Results: The database searches generated 355 results, nine of which were identified as relevant to the aim of this study. In terms of geographical distribution, over half of the studies were conducted in North America, whereas the rest of studies were set in Asia, Europe and Oceania. While almost half of the studies estimated costs associated with IRD in general, others focused on specific conditions, including retinitis pigmentosa (2), Stargardt disease (1) and Choroideremia (1). The majority of studies reported direct medical costs, followed by quality of life, productivity and societal costs.

Conclusion: The small number of results indicate a gap in the literature concerning the economics of IRD. Given the high level of interest around developing innovative therapies for treating these conditions, there is an urgent need for further evidence regarding the current costs associated with IRD, which in turn will help better understand the potential impact of future therapies on these costs for both patients and society.

Characterization of a mouse model for *KCNV2* retinopathy

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Purpose: *KCNV2* retinopathy, characterized by a pathognomonic electroretinogram (ERG) and slowly progressive cone-first photoreceptor degeneration, is a usually recessively inherited retinal disorder, caused by mutations in the *KCNV2* gene. Other symptoms include loss of visual acuity, abnormal color vision and photophobia. One of the most common mutations leading to *KCNV2* retinopathy is a nonsense mutation at position 143, where glutamate is mutated to a premature STOP codon. In order to have a mouse model that would allow studying the pathophysiology of the disease and validating novel treatment approaches, we have generated and characterized a mouse line, where glutamate in position 151 (orthologous to E143 in the human protein) has been mutated to a STOP codon (KCNV2E151X).

Methods: To examine the effects of loss of Kv8.2 in the morphology of the retina, immunohistochemistry experiments were conducted on retinal cryosections of KCNV2$^{E151X/E151X}$ mice and wild-type litter mates of different ages. The sections were stained for DAPI, glial fibrillary protein (GFAP), cone arrestin (CArr) and peanut agglutinin (PNA) and visualized using a Zeiss LSM 710 confocal microscope, to evaluate the time course of photoreceptor loss, retinal damage and cones integrity.

Results: Similar to the clinical observations in human patients, KCNV2$^{E151X/E151X}$ mice show photoreceptor degeneration, as indicated by the reduced number of photoreceptor nuclei, compared to mice that express the wildtype version of Kv8.2 protein (WT). The outer nuclear layer (ONL) is slightly thinner in 4-weeks-old knockout mouse and continues to become thinner with age, indicating not only an early onset, but also a progressive photoreceptor degeneration. GFAP, an indicator Müller glia activation, is increased of KCNV2$^{E151X/E151X}$ mice compared to WT from early on, confirming early onset of the degenerative process. In addition, a progressive loss of cone photoreceptors CArr immunoreactivity in KCNV2$^{E151X/E151X}$ mice can be observed.

Conclusion:
Altogether, these results suggest that KCNV2$^{E151X/E151X}$ mice structurally replicate key features of human *KCNV2* retinopathy. Nevertheless, rod loss appears to be more pronounced in the mouse model. In-depth studies of this mouse line will enhance our understanding of the mechanisms underlying this inherited blinding disease and enable testing of novel therapeutic strategies.

Control of HIF-1α/VEGFA mRNA transcription by miR-205-5p in diabetic mice

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Purpose: Elevated glucose levels in diabetes contribute to oxidative stress and vascular issues, leading to complications such as nephropathy, heart failure, and vision loss. Diabetic patients often exhibit abnormal vascular growth in the retina, potentially triggered by the activation of the HIF-1α/VEGFA mRNA pathway. We propose that miR-205-5p, a microRNA known for its gene regulatory functions and angiogenesis inhibition, could play a significant role in combating proliferative vascular conditions.

Methods: Diabetes was induced in male SWISS mice using Alloxan Monohydrate (200mg/kg). Weekly measurements of blood glucose and body weight were taken. Four weeks post-diabetes induction, the right eye of both control and diabetic mice (n = 20) received an intravitreal injection of a miR-205-5p miRNA mimic, while the left eye served as a control. Mice were euthanized 48 hours post-injection, and a glycated hemoglobin detection test was performed. The eyes were then enucleated, lenses removed, and RNA extracted. The expression profiles of miRNA and mRNA were analyzed using quantitative real-time PCR (qRT-PCR). Data are presented as mean ± SEM (n ≥ 7), with p-values determined by T-Test and ANOVA *p<0.05, **p<0.01, ***p<0.001, and ****p<0.00001.

Results: Induction of diabetes led to weight loss (36.08±0.82), elevated blood glucose levels (>500mg/dl) (547.5±13.18), and a significant increase in glycated hemoglobin levels (12.22±0.46), showing a strong positive correlation among these factors. Additionally, eyes from diabetic mice showed a marked decrease in miR-205-5p levels (0.55±0.08) and a significant increase of HIF-1α and VEGFA mRNA levels compared to controls (8.92±1.60 and 8.06±1.22, respectively). The intravitreal injection of miR-205-5p mimic in diabetic mice normalized the levels of miR-205-5p (3.10±0.73), HIF-1α (2.87±0.42), and VEGFA mRNA (2.69±0.43) to those of controls.

Conclusion: The initial findings indicate that miR-205-5p can mitigate neurovascular proliferation by modulating HIF-1α/VEGFA mRNA levels, highlighting its potential as an alternative therapeutic approach to VEGF antibody treatments in vascular diseases related to the eye.

Ussing chamber system to test the transepithelial resistance of the retinal pigment epithelium in mice

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Purpose: In the eye, the outer blood retina barrier (oBRB) comprises the choroid, Bruch's membrane, and the retinal pigment epithelium (RPE). It plays a crucial role in facilitating the movement of solutes and nutrients from the choroid to the sub-retinal space. In particular, the tight junctions between the RPE cells are important for maintaining oBRB integrity. In retinitis pigmentosa, the most prevalent progressive hereditary retinal degeneration leading to blindness, these tight junctions are altered, potentially leading to fluid leakage and inflammation in the neural retina. As an epithelial cell monolayer, the integrity of the RPE layer can be assessed using transepithelial electrical resistance (TEER) measurements. However, this technique is currently limited to cultured mouse RPE cells. Therefore, we developed a sclera-oBRB-retina preparation and modified an Ussing chamber system for TEER measurements in intact mouse tissue.

Methods: We dark-adapted wild-type C57BL6 mice (aged 4-5 months) prior to dissection, then enucleated the eye and dissected it. The tissue was mounted in cartridge with a 2 mm open hole, electrodes were placed, and the TEER was measured at 33 °C while continuously perfusing the tissue with Ringer medium (mM, NaCl 137, KCl 5, $CaCl_2$ 1.8, $MgCl_2$ 1, glucose 10, HEPES 5, titrated with NaOH to pH 7.4). To validate the method, we induced oxidative stress by introducing 1mM H_2O_2 as it can change the TEER in iPSC-derived RPE cells[1].

Results: The TEER of the sclera-oBRB-retina preparation was measured for a period of up to 180 minutes. Initially, TEER was 103±19 Ω/cm^{-2} and stabilized at 110.3 Ω/cm^{-2} over a period between 30 to 110 mins. First experiments show that H_2O_2 leads to an increase in TEER (130.1 Ω/cm^{-2}) in the sclera-oBRB-retina preparation of the mouse.

Conclusion: Our findings showcase a newly developed methodology for quantitative assessment of oBRB integrity in mouse models for retinal degeneration which can be used as a functional readout after therapeutic intervention.

Reference: Chen, L., Perera, N.D., Karoukis, A.J. *et al.* Oxidative stress differentially impacts apical and basolateral secretion of angiogenic factors from human iPSC-derived retinal pigment epithelium cells. *Sci Rep* **12**, 12694 (2022). https://doi.org/10.1038/s41598-022-16701-6

Characterization of co-cultured microglia in human retinal organoids

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Background: Microglia, the resident immune cells of the central nervous system, play a crucial role in maintaining normal human retinal function. During early development, microglia migrate into the retina, adopting a highly branched phenotype while continuously monitoring their surroundings. Human-derived retinal organoids offer a valuable in vitro model for studying retinal pathophysiology. Our study aims to characterize co-cultured microglia within these organoids, comparing them to non-co-culture organoids as a negative control. This investigation seeks to enhance the retinal organoid model for future research.

Method: Our research employed human induced pluripotent stem cell (hiPSC)-derived retinal organoids, wherein we co-cultured the microglia at D155 for varying time spans. We performed cryosections for immunofluorescence staining using IBA1 and CD68 antibodies specifically targeting microglia and VGLUT1 antibody to visualize the plexiform layers. Using a single-blind method, we analyzed the distribution and quantity of microglia within our organoids. As a negative control, we used age-matched organoids that have not been co-cultured with microglia.

Results: As expected, IBA1 and CD68 signals were absent from control organoids without microglia co-culture. In contrast, in microglia-organoid co-cultures, we readily detected IBA1 signals. Using VGLUT1 signals, we precisely define the outer (OPL) and inner plexiform layers (IPL), improving the accuracy of our analysis across four distinct subregions of the organoids. We found migration of microglia from the outer organoid areas towards the core. The accumulation of IBA1-positive cells within the core area correlated with the duration of co-culture. Specifically, the 3-day co-cultures exhibited higher levels than the 10-day co-cultures.

Conclusion: We established a model for tracking microglia in human-derived retinal organoids. By investigating microglia development in the human retina, particularly in comparison to our microglia-organoid co-culture in vitro model, we aim to elucidate how future enhancements to our organoid model impact microglia generation and distribution, but also Müller cell and neuronal maturation including synaptogenesis.

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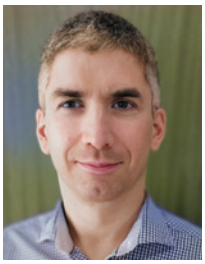

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