PRO RETINA FOUNDATION
FOR PREVENTION BLINDNESS

6th PRO RETINA
Research-Colloquium Potsdam

CONFERENCE REPORT

Retinal Degeneration

10 years into the new century, where do we go from here?

An Interdisciplinary Dialogue

April 9th/ 10th, 2010

Potsdam, Seehotel am Templiner See

supported by
NOVARTIS
OPHTHALMICS

PRO RETINA DEUTSCHLAND E. V. & THE FOUNDATION FOR PREVENTION BLINDNESS

WHO WE ARE

The patient-organisation, "Pro Retina Deutschland e.V." was founded in 1977 as "Deutsche Retinitis Pigmentosa-Vereinigung" by patients and their relatives intended to organize help for themselves. The three objectives mentioned in the constitution are to actively support research, to give psychological and social advice for its members and to strengthen public information.
Every member can join one of the 60 regional groups, which are spread throughout Germany. At present (2010), PRO RETINA Deutschland e. V. counts more than 5,800 members. The Board, the Counsellors, the leaders of the regional groups and all active members are working on a non-profit basis, but they are supported by a fulltime working staff at our office which is located in Aachen (www.pro-retina.de).

WHAT WE DO IN RESEARCH

The jewel of all this work is the Pro Retina-Foundation for Prevention Blindness, which was founded in 1996.
From the early beginning we have created a stable network with researchers and ophthalmologists for joined information and advice. We support research projects with direct financial funding – since the "Foundation for Prevention of Blindness" was established in 1996, more than one million Euro have been donated. We actively initiate research projects and therapy tests and contribute to their implementation.

Every year, we award two research prices and organize and support national and international seminars and conferences on relevant topics. We are financing PhD grants in order to foster research activities and networking between researchers.

We are consulted by a Scientific and Medical Advisory Board ("Wissenschaftlicher und Medizinischer Beirat", WMB) and a Working Group on Clinical Questions ("Arbeitskreis Klinische Fragen", AKF). In this Working Group scientists of different medical and other relevant disciplines are taking part.

The main objective is to secure a long-term support for research activities, e. g. by granting financial means for the development of new research projects or by financing the initial phase of relevant projects.

It is envisaged to increase the capital of the foundation to a minimum of Euro 5,000,000, which are to result in a steady source of funding for the support of research, independent from changing income of donations.
We guarantee that the benefits of the Foundation will only be dedicated to the research of retinal diseases, with the wider objective to develop applicable therapies for the patients.

PROGRAMME

Friday, April 9th 2010

13:00–13:05 **Franz Badura,** PRO RETINA Foundation, research division
Opening remarks

13:05–13:15 **Eva Luise Köhler,** Patron of the Alliance for Chronic Rare Diseases (ACHSE)
"*ACHSE and national research efforts for rare diseases"*

13:15–13:30 **Eberhart Zrenner,** Tübingen, Chairman of the Scientific Medical Advisory Board
"Present concepts for improvement of medical care of rare eye diseases in Germany"

13:30–15:30 **Session 1: Diagnosis**

Chairman: Prof. Klaus W. Rüther

1. Bart Leroy, Ghent: *"Diagnosis by gene, retinal morphology and function"*
2. Hendrik P.N. Scholl, Wilmer Eye Institute, Johns Hopkins University, Baltimore, USA: *"Structure-function correlation of the human central retina"*
3. Christoph Friedburg, Giessen: *"Psychophysics and electrophysiology"*
4. Ulrich Kellner, Siegburg: *"Imaging"*

15:30–16.15 Coffee break and scientific chitchat

16:15–18:15 **Session 2: New mechanisms of degeneration**

Chairman: Prof. Olaf Strauß

1. Christian Grimm, Zürich: *"Degenerative mechanisms"*
2. Andreas Ohlmann, Regensburg: *"Mediators of viability or apoptosis"*
3. John Ash, Oklahoma: *"Cellular interactions: The role of Müller cells"*
4. Sandra Cottet, Sion/Lausanne: *"Cell death pathways in photoreceptor degeneration"*

18:30 Dinner

19:30 **Swingin' poster session and get together**

PROGRAMME

Saturday, April 10th 2010

08:30–10:30 **Session 3: Identification of new disease genes**

Chairman: Prof. Bernhard H. F. Weber

1. Frans Cremers, Nijmegen: *"Novel approaches to gene identification"*
2. Marius Ueffing, Tübingen: *"The potential of proteomics"*
3. Joachim Graw, Helmholtz München: *„Mouse models to identify novel disease genes"*
4. Andreas Gal, Hamburg: *"Genetic modifiers in retinal disease"*

10:30–11:00 Coffee break

11:00–12:30 **Session 4: Therapy**

Chairman: Prof. Marius Ueffing

1. Bill Hauswirth, Gainesville/Florida: *"Gene Therapy – RPE65"*
2. a) Peter Walter, Aachen: *„Epiretinal prosthesis"*
   b) Eberhart Zrenner, Tübingen: *„Subretinal prosthesis"*
3. Frank G. Holz, Bonn: *"Therapeutic targets in geographic atrophy"*

12:30–13:00 **Poster awards and short presentations**

13:00 Lunch and end of meeting

Eva Luise Köhler,
Ehefrau des Bundespräsidenten, Horst Köhler.
Schirmherrin der Allianz Chronisch Seltener Erkrankungen (ACHSE)

wife of the Federal President, Horst Köhler
patron of the Alliance for Chronic Rare Diseases (ACHSE)

SPEAKER ABSTRACTS

Present concepts for improvement of medical care of rare eye diseases in Germany

Eberhart Zrenner

Center for Ophthalmology
Institute for Ophthalmic Research
University of Tübingen

Rare diseases are very common. Thirty million of European citizens suffer from a rare disease. As many primary physicians are not very well acquainted with rare diseases due to low incidence it takes 3.3 years in average until a correct diagnosis has been achieved (Eurordis study 2005). In recent years an alliance for chronic rare diseases (ACHSE e.V.) has been founded in Germany, representing approximately 90 member organizations and approximately 1000 diseases with a million of affected people and supporting the further development of medical care for rare eye diseases.

A recent study of the Ministry of Health in Germany has revealed numerous problems concerning patients with rare disease, such as access to adequate health care providers, lack of accepted recommendations for diagnosis and therapy, missing clinical studies, lack of data bases, missing coordination of research. Therefore a national action forum for rare diseases has been implemented in Germany. A number of tools has been identified already to improve the situation:

- Interdisciplinary networks of specialists for health care for the various rare diseases
- Certified reference centers acting across regional borders
- Special role of university clinics that perform research and highly specialized health care
- Continuing education concerning the various rare diseases
- Establishment of European centers of reference linked to partners in a network for coordination of research and health care
- patient registry
- Biobanking
- Information service
- Development of clinical studies
- Translational research for new therapeutic strategies

The Ministry of Research already fosters presently 15 networks for various rare diseases that aim at improving the situation of patients with rare diseases by harmonized goal oriented actions. It will be very important to improve the situation by speeding up proper diagnosis for patients, developing and financing of reference centers for rare diseases, fostering informa-

tion transfer between the various stake holders, advancing implementation of research and innovation, improvement of health care access and connection to international networks for the various rare diseases. It will be very crucial to achieve such goals in order to improve the situation for health care, research and continuing education in the field of rare eye diseases.

"Diagnosis by gene, retinal morphology and function"

Bart P Leroy[2]

[1]Department of Ophthalmology & [2]Center for Medical Genetics, Ghent University Hospital, Ghent, BELGIUM,

Purpose: To describe how the specific aspects of phenotypes and genotypes of retinal dystrophies can contribute towards making the correct diagnosis.

Methods: A case presentation format will be used to illustrate ocular and sometimes systemic clues that help in making a specific diagnosis in different genetically determined retinal conditions. Both clinical and electrophysiological phenotypes as well as genotypes will be discussed.

Results: Phenotypes and genotypes of genetically determined diseases leading to progressive visual loss are very different. An important distinction to be made is the one between stationary and progressive diseases. This is essential in order to identify whether or not the condition is likely to evolve over time. Further, within the group of dystrophies, with by definition progression of disease, the rate of visual loss and the final visual outcome can be very different between different conditions.

Conclusions: Clues which help to make a specific diagnosis can be of three different kinds: clinical, electrophysiological or molecular. Differences between clinical phenotypes, both at the ocular and systemic level, can sometimes help to lead to the identification of a gene, mutations in which are the underlying cause of disease. Likewise, specific phenotypic clues can sometimes be obtained by visual electrophysiology. In addition, different mutations in one and the same gene may lead to very diverse phenotypes, each with a different prognosis.

Structure-function correlation of the human central retina

Peter Charbel Issa [1,2], Eric Troeger [3], Robert Finger [1], Frank G. Holz [1], Robert Wilke [3,4], Hendrik P.N. Scholl [1,5]

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Abstract: The impact of retinal pathology detected by high-resolution imaging on vision remains largely unexplored. Therefore, the aim of the study was to achieve high-resolution structure-function correlation of the human macula in vivo. To obtain high-resolution tomographic and topographic images of the macula spectral-domain optical coherence tomography (SD-OCT) and confocal scanning laser ophthalmoscopy (cSLO), respectively, were used. Functional mapping of the macula was obtained by using fundus-controlled microperimetry. Custom software allowed for co-registration of the fundus mapped microperimetry coordinates with both SD-OCT and cSLO datasets. The method was applied in a cross-sectional observational study of retinal diseases and in a clinical trial investigating the effectiveness of intravitreal ranibizumab in macular telangietasia type 2. There was a significant relationship between outer retinal thickness and retinal sensitivity ($p<0.001$) and neurodegeneration leaving less than about 50 µm of parafoveal outer retinal thickness completely abolished light sensitivity. In contrast, functional preservation was found if neurodegeneration spared the photoreceptors, but caused quite extensive disruption of the inner retina. Longitudinal data revealed that small lesions affecting the photoreceptor layer typically precede functional detection but later cause severe loss of light sensitivity. Ranibizumab was shown to be ineffective to prevent such functional loss in macular telangietasia type 2. Since there is a general need for efficient monitoring the effectiveness of therapy in neurodegenerative diseases of the retina and since SD-OCT imaging is becoming more widely available, surrogate endpoints derived from such structure-function correlation may become highly relevant in future clinical trials.

Psychophysics and electrophysiology in retinal disease with a focus on children

C. Friedburg and B. Lorenz

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Purpose: To provide an overview and perspective of the role of psychophysics and electrophysiology in the diagnostics of retinal disease with a focus on children.

Methods: After an introduction to psychophysical approaches, the range of available psychophysical tests is described including specifically manual / computer perimetry under light and dark adaptation. Children pose a special challenge regarding cooperation, focussing attention and response to tasks. Special techniques, e.g. controlling fixation, may reduce some of these problems. We present a study on fundus-controlled perimetry in 40 children, aged 4 to14 y (median 7 y). Electrophysiology is equally challenging in children. The use of visual evoked potentials (VEP), electroretinogram (ERG), multifocal (mf-) ERG and electrooculogram (EOG) is reviewed with special reference to the patient's age.

Results: Age-adapted sets of investigations combining psychophysical and electrophysiological methods provide, together with imaging, a quantitative measure of certain aspects of vision important in retinal disease. The reliability of both psychophysics and electrophysiology largely depends on the expertise of the examiner and the communication with the patient: Pattern VEPs and ERGs may be obtained in an awake toddler or a five year-old child. An elaborate sequence of tests and specialized ophthalmological centers are essential to provide reliable diagnostics, to keep evaluation cost-effective and to avoid unnecessary over-diagnosing with MRI or even CSF analysis in children with unexplained visual "loss" or nystagmus. The quantitative measures are an important puzzle piece to distinguish progressive from stationary disease, to inform patients and their families about prognosis and recurrence risk, and to evaluate eventually available treatments of retinal disease. Specific patterns of results e.g. the amount of cone and rod involvement or negative or hypersensitive ERGs can guide molecular diagnosis. Methods like fundus-controlled perimetry improve control of retinal location of stimulation and are important especially in macular disease and its differential diagnoses in children.

Conclusion: A whole battery of psychophysical and electrophysiological methods is now available and can be used in a significant number of children when dedicated examiners perform the test. Thus, a precise clinical diagnosis can be obtained even in young children. This is important in order to provide parents with the necessary information as to the nature of the disease in their child, to optimise the overall development in the presence of a visual handicap, and to identify children that may profit from therapeutic approaches that have been developed recently or will become available in the near future.

Visualizing retinal disease processes: Comparative retinal imaging

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Purpose: To report recent developments in advanced retinal imaging to visualize retinal disease processes

Methods: Using a widely available scanning laser ophthalmoscope (Spectralis HRA & OCT, Heidelberg Engineering) with two different laser wavelengths for illumination and different settings for filtering the emitted light four non-invasive imaging methods are available: red-free imaging (RF), near-infrared reflectance (NIR), fundus autofluorescence (FAF), near-infrared-autofluorescence (NIA). In addition, high resolution optical coherence tomography (OCT) is available.

Results: Whereas NIR and OCT detect alterations in different retinal layers, RF allows to selectively analyze epiretinal alterations, whereas FAF and NIA focus on the lipofuscin (FAF) and the melanin (NIA) in the retinal pigment epithelium (RPE). The combined use of these techniques allows a detailed analysis of the development of retinal disease processes in inherited and acquired disorders.

Conclusion: Comparative retinal imaging is important for understanding retinal disease processes and for the detection of early stages of retinal disease. This will be helpful for the development and application of future therapies.

Degenerative mechanisms in retinal degeneration

Christian Grimm

Lab for Retinal Cell Biology, Dept Ophthalmology and Center for Integrative Human Physiology, University of Zurich, Zurich, Switzerland

Purpose: To develop efficient therapies for retinal degenerations, it is important to understand the molecular mechanisms inducing and executing cell death in the retina. We use several inducible and inherited mouse models of photoreceptor degeneration to investigate physiology and pathophysiology of the retina and to identify endogenous survival systems which may protect photoreceptors from degeneration.

Methods: Wild type mice are exposed to high levels of visible light to induce photoreceptor degeneration. Animals of models for inherited retinal degeneration are analyzed at different timepoints during disease progression. Gene expression is studied by real-time PCR, Western blotting, in situ hybridization and immunofluorescence. For hypoxic preconditioning, mice are exposed for 6 h to reduced levels of oxygen. Tissue-specific gene knockdowns are achieved using the cre-lox system.

Results: Hypoxic preconditioning activates several transcription factors including HIF-1a, HIF-2a and STAT3, leading to a differential regulation of the respective target genes and to the protection of photoreceptors from light damage. Knockdown experiments showed that photoreceptor specific expression of HIF-1a is not required for protection. Photoreceptor specific knockdown of 'von Hippel Lindau' protein prevents degradation of HIF-alpha subunits, activates STAT3 and leads to a gene expression pattern which partially mimics the retinal response to hypoxia. However, protection of photoreceptors is only transient. This suggests that full protection, as induced by hypoxic preconditioning, requires additional factors and / or the involvement of retinal cell types in addition to photoreceptors.
Photoreceptor stress or injury induces expression of leukemia inhibitory factor (LIF) in a subset of Muller glia cells. LIF controls an elaborate molecular defense system which is activated in all models of retinal degeneration analyzed so far. This system includes increased expression of endothellin-2, GFAP and fibroblast growth factor-2 and leads to an increased survival of photoreceptors. In the absence of LIF, this system is not activated, Muller cells remain quiescent and retinal degeneration shows an increased severity.

Conclusion: Retinal stress like hypoxia or photoreceptor injury can induce endogenous survival systems to protect photoreceptors from cell death. Although the molecular response of photoreceptors contributes to survival in such situations, Muller glia cells seem central for maintaining photoreceptor integrity in the injured retina. Exogenous regulation and support of endogenous survival pathways may proof beneficial for the preservation of vision in degenerative diseases.

Norrin Mediates Neuroprotective Effects on Retinal Neurons via Activation of the Wnt/β-Catenin Pathway and the Induction of Neurotrophic Growth Factors

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Norrin is a secreted protein that activates Wnt/β-Catenin signaling and is part of an essential signaling system that controls the formation of retinal capillaries during development. Since Norrin-deficient mice show an early specific loss of retinal ganglion cells (RGC), we hypothesized an additional neuroprotective role of Norrin. To analyze this function of Norrin in more detail, we investigated its neuroprotective properties on RGC survival in mouse eyes. To induce RGC damage, N-methyl-D-aspartate (NMDA) was injected into the vitreous body of one eye while the fellow eye received NMDA in combination with Norrin and/or Dickkopf (DKK)-1, an inhibitor of Wnt/β-Catenin signaling. After injection of NMDA, the numbers of optic nerve axons and of perikarya of surviving RGC were significantly higher in NMDA/Norrin injected eyes as compared to NMDA treated eyes, an effect that could be blocked with DKK-1. Comparable results were obtained by TUNEL labeling. After treatment of cultured Müller cells with Norrin or of mouse eyes with NMDA plus Norrin, the levels of β-Catenin were markedly increased, indicating an activation of the Wnt/β-Catenin signaling pathway. In addition, Norrin induced an activation of Müller cells and the expression of Lif and endothelin-2 after retinal damage with NMDA. Moreover, Norrin induced the expression of neuroprotective growth factors such as FGF2, BDNF, LEDGF and CNTF in damaged retinae and cultured Müller cells. We conclude that Norrin has pronounced neuroprotective properties on RGC via activation of Wnt/β-Catenin signaling and subsequent induction of neurotrophic growth factors in Müller cells.

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Cellular interaction: the role of Muller cells

John D. Ash

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Purpose: Muller cells have repeatedly been shown to activate in response to retinal injury and inherited retinal degeneration. As part of that response, we and others have shown that there is a dramatic elevation of neuroprotective cytokines including LIF, BDNF, End2 (endothelin 2) and FGF2 during chronic stress. Published data suggests that reactive Muller cells produce neuroprotective factors which serve to delay or prevent neuronal cell death. We have found that gp130 activation, a common signal-transducing receptor used by LIF, is essential for stress-induced endogenous protection of photoreceptors. We have also found that Gp130 signaling through the Jak/STAT3 pathway in photoreceptors is necessary for protection. Once activated, STAT3 is a nuclear transcription factor. We have hypothesized that STAT3 is protecting photoreceptors through increased transcription of protective genes.

Methods: Our comparison groups were mice injected with low doses of LIF that protect photoreceptors from light damage without inhibiting light responses. We have conducted DNA microarray and chromosomal immunoprecipitation assays to identify genes and pathways regulated by STAT3 activation.

Results: Our data suggest that activated STAT3 elevates the expression of End2 and FGF2. However, cell specific knockouts were used to show that End2 is elevated in photoreceptors which is associated with protection, but FGF2 is elevated in Muller cells which is not associated with protection. Data also show that STAT3 elevates the expression of anti-apoptotic genes, and induces the expression of an interferon gamma response pathway.

Conclusion: This study provides direct evidence that Muller cells stimulate endogenous protection of photoreceptors through elevated expression of ligands which activate gp130/STAT3 in photoreceptors. gp130-STAT3 activation in photoreceptors increases the expression of multiple protective pathways include those that protect mitochondrial function and modulate immune responses.

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Bax-induced apoptosis in Leber's congenital amaurosis: a dual role in rod and cone degeneration

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Purpose: Pathogenesis in the *Rpe65*$^{-/-}$ mouse model of Leber's congenital amaurosis (LCA) is characterized by a progressive loss of the rods, while cones degenerate rapidly at early ages. Retinal degeneration in *Rpe65*$^{-/-}$ mice was previously reported to depend on continuous activation of a residual transduction cascade by unliganded opsin. However, the mechanisms of apoptotic signals triggered by abnormal phototransduction remain elusive. We therefore assessed whether activation of Bcl-2-mediated apoptotic pathway was dependent on constitutive activation of the visual cascade through opsin apoprotein. We further challenged the direct role of pro-apoptotic Bax protein in triggering apoptosis of rod and cone photoreceptors.

Methods: Quantitative PCR, Western blot and immunohistological analyses were performed to monitor regulated expression of apoptotic and photoreceptor genes during the course of the disease. Apoptosis and loss of photoreceptors were assessed by TUNEL assay and histology.

Results: Triggering of Bcl-2-related apoptotic pathway in *Rpe65*$^{-/-}$ mice was associated with photoreceptor apoptosis, as reflected by altered expression of Bcl-2-related proteins and activation of pro-apoptotic Bax. Imbalanced Bcl-2/Bax expression was restored and photoreceptor apoptosis prevented in phototransduction-deficient *Rpe65*$^{-/-}$ / *Gnat1*$^{-/-}$ mice, indicating that abnormal activity of opsin apoprotein induces apoptosis through the Bcl-2-mediated pathway. We further observed in *Rpe65*$^{-/-}$ / *Bax*$^{-/-}$ mice that decreased expression of rod genes as well as apoptosis were rescued, confirming that rod degeneration is dependent on Bax-induced pathway. Surprisingly, early loss of cones was not prevented in *Rpe65*$^{-/-}$ / *Bax*$^{-/-}$ mice, indicating that Bax is not involved in cone cell death in *Rpe65*-LCA disease.

Conclusion: These observations demonstrate that in *Rpe65*-LCA disease a single genetic mutation can trigger two independent degenerative pathways in rods and cones and that a Bax-independent, cell autonomous mechanism is involved in cone cell death. They furthermore highlight the necessity to investigate specific death signaling pathways committed in rods and cones to develop effective therapeutic approaches to treat RP diseases.

Novel approaches to retinal disease gene identification

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Purpose: Inherited retinal diseases display a high degree of genetic heterogeneity, which renders the identification of the underlying disease genes very challenging. We develop novel genomics strategies to identify these genes.

Methods: The generally low prevalence of retinal disease-associated mutations can be used to our advantage as these mutations often occur homozygously in patients. By using single nucleotide polymorphism (SNP) arrays, we can identify the regions encompassing these mutations, both in consanguineous and nonconsanguineous families. The identification of causal mutations is straigthforward if attractive candidate genes reside in the region of interest, but can be tedious if these are missing. With the introduction of next generation sequencing, this hurdle can now be readily taken.

Results: In the Dutch population, ~1/3 of patients with inherited retinal diseases carry homozygous mutations. This suggests that the Dutch population contains subpopulations that until two generations ago, were relatively isolated. We performed genome-wide homozygosity mapping using high-density SNP arrays in ~400 patients with autosomal recessive retinitis pigmentosa (RP), Leber congenital amaurosis (LCA), cone- or cone-rod dystrophy (CD, CRD), or achromatopsia (ACHM). Patients were ascertained predominantly in the Netherlands, Canada, and Germany. These studies revealed many patients from nonconsanguineous families with conspicuous homozygous regions. By employing bioinformatic tools, we identified the LCA gene *LCA5*, the arRP gene *EYS*, and the CD/ACHM gene *PDE6C*. In many patients however, the homozygous regions encompass hundreds of genes without obvious candidates.
Recently, next generation sequencing enabled us to analyse 330 genes in a 40-Mb chromosomal region that cosegregated with familial exudative vitreoretinopathy (FEVR) in two sizeable families. A missense variant was identified in *TSPAN12* in both families. In total we identified *TSPAN12* mutations in 11 families with FEVR.

Discussion: Homozygosity mapping in both consanguineous and nonconsanguineous families has proven to be effective in identifying novel retinal disease genes. Next generation sequencing represents a powerful technology that will facilitate the identification of the remaining retinal disease genes in the next few years.

The potential of proteomics: affinity based approaches to study protein networks in retinal disease

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Purpose: Understanding the molecular mechanisms of diseases is one of the big challenges in modern biology. In spite of the abundance of genetic, clinical and experimental data, information on the molecular representation of disease is scarce. It remains rather enigmatic, on how specific gene variants or mutations affect the healthy balance of an organism. The analysis of protein networks can facilitate an in depth understanding on the pathological mechanisms of disease. Proteomic as well as reverse genetic experimental techniques, each with their own inherent experimental errors, have evolved to study protein networks, yet quantitative methods are needed to determine the specific impact of mutation on a systemic level.

Methods: Towards this aim, we generated a workflow to enable quantitative and comparative assessment of changes in interaction patterns caused by mutation. Tandem affinity purification (TAP) employing a new tandem affinity purification (TAP) strategy, developed by us (*Gloeckner et al., Proteomics 2007)* is combined with stable-isotope labeling by amino acids in cell culture (SILAC) and subsequent mass-spectrometric analysis. The resulting workflow allows quantitative comparison of changes within a protein complex of interest as a consequence of allelic variance or mutation. Besides revealing mutation specific features, this method is generally applicable to quantitatively analyse changes in protein–protein interactions due to genetic, physiological or environmental perturbations.

Results: We have analysed a severe form of blindness, Leber congenital amaulrosis (LCA). Mutations leading to LCA specifically affect the function of photoreceptor cells in the retina. Comparing protein interactions mainly by tandem affinity purification of normal or a corresponding mutant allele we have analysed the interactomes of of normal versus mutant lebercilin, which is genetically linked to LCA5 (*Den Hollander et al., Nature Genetics 2007*). For Lebercilin, description of its protein network revealed protein modules of the interflagellar transport (IFT) machinery as main interactors. IFT specifically supports selective protein transport from the inner segment of the cell to the outer segment, which is the light sensitive cellular structure in mammalian eyes. This cellular structure is maintained through directed protein

transport. Quantitative SILAC comparisons of mutated versus normal allele show that specific functional modules of the IFT machinery equivalent to specific components of this molecular machine disconnect as a consequence of lebercilin's mutation, whereas other functional connections are not affected at all. Changes can be accurately and reproducibly quantified showing the impact of a discrete mutation in a specific protein on the level of an entire protein network.

Mouse models to identify novel disease genes

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Purpose: To identify novel disease genes responsible for eye disorders in the mouse.

Methods: Male C3HeB/FeJ or C57BL/6J mice were treated with N-ethyl-N-nitrosourea (ENU) and mated with untreated females; the offspring were screened for gross morphological alterations including the eye by manual inspection or for ocular disorders by slit lamp, funduscopy and laser interference biometry (LIB). In confirmed mutant lines, we performed a genome-wide linkage analysis and sequenced positional candidate genes. The second approach characterizes already existing mouse mutants by comprehensive phenotyping within the framework of the German Mouse Clinic (GMC).

Results: In the ongoing ENU screen, a total of 1700 C57BL/6J mice have been screened. The most efficient screening method was LIB leading to 12 new mouse mutant lines. 3 lines were mapped, and their underlying mutations are identified: *Aca23* suffers from a small cornea and an enlarged anterior chamber; the mutation affects *Col8a2*, which is known to be involved in corneal disorders. *Aca12* is characterized by a small lens and shorter axis; the underlying mutation affects Fgf9, which was not yet reported to be affected in eye disorders. *Aca30* has a similar phenotype like *Aca12*, but the underlying mutation affects the *Cryba2* gene; there is no mutation reported neither in mice nor in humans.
Screening by funduscopy revealed 2 confirmed mutant lines. *Fun6* mutants are characterized by optic disc anomalies; the mutation is localized on chromosome 19. *Fun22* mutants show a pale spotted fundus.
Among the C3HeB/FeJ-treated mice, *Aey69* mutants are suffering from anophthalmia/microphthalmia; the mutation is mapped to chromosome 3.
The ocular phenotype of the *Ali30* mutant was picked up in the GMC: it is suffering from optic nerve head dysplasia and characterized by a splice-site mutation in the *Bmpr1b* gene, which was not yet reported to be responsible for ocular disorders.

Discussion: The outcome of screening methods depends not only on the numbers of screened animals, but also on the applied screening methods. Additional screening techniques resulting in quantitative data (Scheimpflug imaging for lens opacities) or in higher resolution (like optical coherence tomography for retinal disorders) are necessary. Subtle phenotypes in young animals might be important indicators for significant age-related disorders.

Genetic modifiers of the phenotype in retinal disease

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Heterogeneity is a common feature of human genetic diseases. Genetic heterogeneity consists of allelic heterogeneity, in which different mutations in one gene result in the same trait, and locus heterogeneity, in which mutations in two or more genes produce the same phenotype. Clinical heterogeneity refers to largely variable phenotypes that can be observed in patients with the same disease. This phenotypic variability, both among patients from the same family and in unrelated patients affected by the same disease, seems to be, at least in part, due to genetic modifiers. Modifier genes alter the phenotype produced by another gene, due to their effects on penetrance, dominance, age of onset, progression, expressivity, or pleiotropy. Usually there is a single modifier allele that is not highly evolutionarily conserved and has a modest effect. Modifier genes may qualitatively or quantitatively alter the phenotype, and their effect can be observed if they are present or absent. In the case of penetrance, the modifier may change the frequency of affecteds among mutant homozygotes or heterozygotes by moving the threshold for expressing the trait, i.e. a greater or smaller proportion will be affected. Variable expressivity, the extent/severity of the trait in affecteds, can be explained by a modifier that shifts the distribution for mutant homozygotes (or heterozygotes) relative to the phenotypic threshold. Dominance of an allele can be altered by a modifier through moving the threshold for expressing the trait, i.e. heterozygotes are (or are no longer) affected. In my presentation, I will review examples from the literature on modifier alleles affecting various genetic features and the molecular mechanisms of their action in various retinal diseases.
Modifier genes may act in both directions and as such may represent susceptibility alleles, that increase disease risk, or protective alleles, that reduce it. The latter may be promising targets to study in order to better understand 'natural genetic resistance to disease' and develop strategies to exploit it pharmacologically.

Leber congenital amaurosis gene therapy clinical trial

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Purpose: Results of a recently initiated AAV vector clinical trial for LCA2 (recessive RPE65 mutations) will be discussed.

Methods: Each of three LCA2 patients received 150ul (5.9 exp10 vector genomes) of GMP grade AAV2-CBA-hRPE65 vector subretinally and their visual function then followed periodically over the next year.

Results: At 3 months post-treatment no adverse events were noted for any patient. All three also demonstrated substantial and significant improvement in light sensitivity, up to 63,000 times better that pretreatment baselines, but only in the area of retina that received vector. Two of the three patients experienced full recovery of retinal function within the vector treated area. At one year post-treatment, all vision improvements remained stable and no adverse events were noted. However, one patient reported new visual perceptions at 12 months post-treatment but not before. When asked to detect a very bright target that she could see before treatment she continued to use her fovea. Paradoxically, if she was asked to detect a dim target that she could not previously detect at any time up to 9 months post-treatment, she could now see it. When her visual fixation was analyzed the patient was found to have shifted her center of visual fixation away from her fovea into the treated retinal area. In effect, she had developed a second fovea or "pseudo-fovea" that was used only when the target was too dim to be perceived by her anatomical fovea. Determination of the retinal topography of cone sensitivity confirmed that the patient had not lost her foveal sensitivity but had gained an extra-foveal area of light sensitivity corresponding to the vector treated area.

Conclusion: This slow emergence of a pseudo-fovea suggests that cortical "learning" is possible but slow in a young adult when vision is restored by gene therapy.

The epiret retinal prosthesis – results from clinical observations

Peter Walter

RWTH Aachen

Introduction: RP is currently untreatable. Visual prostheses may provide a tool for stimulation of retinal neurons. Several approaches are under investigation among them epiretinal and subretinal devices. Data from clinical trials confirm the proof of principle. We performed a study on six blind RP subjects with a four week implantation period, three sessions of stimulation and a long-term follow-up after explantation.

Materials and Methods: Six blind RP patients underwent surgery for implantation of the EPIRET3 device which is fully implanted without any cable connections crossing the eye wall. Adverse events were carefully documented throughout the follow-up. Stimulation data were obtained for threshold and subjective reports were recorded. After explantation patients were followed for five months during the prospective trial. Patients were seen in an extension study for 2 years after explantation.

Results: Surgery was successful in all patients. Adverse events were a pseudoendophthalmitis in one case after implantation and a retinal break during explantation requiring specific further surgery. After 2 years all eyes are stable, the patients had no change in quality of life scores and all patients wanted to continue with the research programme.

Conclusion: The EPIRET 3 device is safe and is the only device world wide offering a full implantation within the eye. Next steps are to increase the number of stimulating electrodes in a fourth generation implant.

Subretinal Prosthesis: Possibilities of restitution of vision in patients blind from hereditary retinal degeneration

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Purpose: Restoration of letter reading and stripe pattern recognition via subretinal electronic implants in blind RP patients.

Methods: Subretinal implants were placed transchoroidally near the macula, consisting of two arrays: 4 x 4 electrodes (100 x 100 µm), spaced 280 µm, controlled retroauricularly via a subdermal line for direct stimulation ("DS array") and a "chip" (3 x 3 x 0,1 mm) with 1500 electrodes (50 x 50 µm) of the same kind,each electrode being activated by light falling onto a neighboring microphotodiode that controls the output of its subretinal amplifier. Letters were presented to 3 patients either by stimulating retinal cells in 10ms steps via individual electrodes in a sequence patients had learned to write such letters or - via the light sensitive chip -by individual letters or stripe patterns steadily presented at a screen.

Results: On the DS array patients reported uniformly for each electrode that the sensation evoked by each individual pulse (0.5 - 4 ms, 0.1 V above threshold) consisted of a whitish round dot, clearly separated from its neighbor. Patterns consisting of such 4 x 4 dots correspond to letters of approximately 5 cm diameter presented at 60 cm distance. Pat. 1 correctly (20/24) recognized the direction of the letter "U", presented with the opening in four different directions in in a 4 alternative forced choice (4AFC) mode. Pat. 2 correctly (12/12) differentiated letters (e.g. C, O, I, L, Z, V) within few seconds, presented via DSelectrodes in random order (4AFC). With the light sensitive subretinal chip, he also correctly (22/24) differentiated without head movements letters (e.g. L,I,T,Z; 8,5 cm high, 1.7 cm line width) steadily presented on a screen at 62 cm distance with a red light (630nm cutoff) of 3.4 cd/m^2. Pat. 3 recognized (15/20 correct, 4AFC) the direction of lines or stripe patterns with the chip, as did Pat. 1 (11/14, 2AFC) and Pat.2 (11/12 4AFC) up to 0.35 cycles/deg.

Conclusions: Active subretinal multielectrode implants with currents close to recognition threshold (10 to 27 nC/electrode) produce retinotopically correct patterns that allow for the first time recognition of relatively small individual letters (8cm high, viewed in appr. 62 cm distance) even at low luminance levels. Stripe patterns of moderate luminance can be resolved up to 0.35 cycles/deg via the subretinal chip. This clearly supports the feasibility of light sensitive subretinal multi-electrode devices for restoration of useful visual percepts in blind patients.

Therapeutic targets in geographic atrophy

Frank G. Holz

Geographic atrophy (GA) - a 'late-stage' manifestation of complex, multifactorial age-related macular degeneration (AMD) - represents a common cause for severe visual loss with increasing prevalence and incidence. While neovascular AMD can now be effectively treated with VEGF-neutralizing humanized antibodies, GA represents a large unmet clinical need with no proven treatment yet. Cell death in GA occurs in corresponding outer neurosensory retinal layers, the RPE and the choriocapillaris and lesions show a continuous enlargement over time. No exclusive dichotomy exists for neovascular or atrophic forms of AMD and both phenotypic manifestations appear to share the same known genetic risk factors. Although the pathogenesis of GA is incompletely understood, the understanding of relevant pathways has been expanded over recent years. Several lines of evidence from basic and clinical science indicate the relevance of excessive lipofuscin (LF) accumulation in the lysosomal compartment in postmitotic RPE cells with age and disease, whereby built-up of LF granules represent a common downstream pathogenetic pathway in various hereditary and complex retinal diseases. Toxic LF-compounds including A2-E have been identified and molecular mechanisms of their interference with normal RPE functions have been elucidated including inhibitiaon of the lysosmal proton pump and subsequent impairment of lysosomal degradation. With the advent of confocal scanning laser ophthalmoscopy (cSLO) fundus autofluorescence (FAF) imaging now allows for detection and topographic mapping of intrinsic fluorophores including A2-E in vivo. In GA it has been demonstrated that atrophic lesions develop and expand, respectively, in areas with increased FAF-signals at baseline. These observations formed the rationale to develop and test pharmacological agents that antagonize the accumulation of toxic byproducts of the visual cycle, e.g. by reducing the amount of retinoid-precurses, or by direct inhibition of visual cycle-specific enzymes. Further attempts to modulate the disease process include the intraocular delivery of CNTF or the application of a serotonin 1 agonist, as well as neuroprotective and antiapoptotic agents. Should clinical efficacy and safety be demonstrated, the potential application in early AMD to slow progression of disease before visual loss occurs as well as use in other retinal disease entities that share pathogenetic pathways of AMD may be sought.

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POSTER ABSTRACTS

Mutual inhibition of Norrin and TGF-β in vitro and in vivo

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Background: Norrin is an angiogenic and neuroprotective growth factor that can activate the canonical Wnt/β-catenin pathway via binding to the frizzled-4 receptor. The predicted tertiary protein structure of Norrin has similarities to that of transforming growth factor (TGF)-β1 and other growth factors containing a cysteine knot motive. Therefore we analyze, whether Norrin can interact with the TGF-β1 signaling pathway and vice versa in vitro and in vivo.

Methods: Mink lung epithelial cells (MLEC) and human microvascular endothelial cells (HMEC) were studied. MLEC had been stably transfected with the coding sequences of luciferase under control of the TGF-β responsive promoter fragment of plasminogen activator inhibitor (PAI) and served as reporter for TGF-β1 signaling. MLEC and HMEC were incubated with Norrin, Dickkopf (DKK)-1 and/or TGF-β1 to analyze luciferase activity, expression of PAI-1, TGF-β1, Smad7, TGF-β-Receptor 1 (TGFβ-R1), as well as cell proliferation. In parallel experiments, transgenic mice with an ocular overexpression of Norrin (βB1-Norrin; Ohlmann et al., *J. Neurosci.* 2005) or TGF-β1 (βB1-TGF-β1; Flügel-Koch et al., *Dev. Dyn.* 2002) were mated and double transgenic mice were examined by light and electron microscopy, and real-time RT-PCR.

Results: In TGF-β1 treated MLEC a substantial increase in the activity of luciferase was observed, an effect that was significantly reduced when TGF-β1 was added in combination with Norrin. The inhibitory effect of combined Norrin/TGF-β1 was completely blocked after adding DKK-1, an inhibitor of canonical Wnt/β-catenin signaling. HMEC that were incubated with TGF-β1 showed an increase in the expression of PAI mRNA, an effect that substantially decreased upon combined treatment with Norrin/TGF-β1. After incubation of HMEC with Norrin alone, a pronounced decrease in mRNA for TGF-β1, TGFβ-R1 was observed, whereas the expression of Smad7, an inhibitor of TGF-β signaling is induced. On the other hand treatment of HMEC with TGF-β1 reduced the Norrin-mediated increase in proliferation by about 23%.
In retinae of transgenic βB1-TGF-β1 mice the expression of norrin mRNA was significantly decreased when compared to that of wildtype littermates. Vice versa, in retinae of βB1-Norrin mice the expression of TGF-β2 mRNA was significantly lower than in wildtype littermates. Interestingly, in both transgenic mouse lines the level of Smad7 mRNA was decreased in retina, when compared with wild type controls, but returned to normal levels in double transgenic mice. Finally, the ocular phenotype of βB1-TGF-β1 mice such as lack of vitreous body, increase of apoptotic neurons in the retina, and the absence of retinal capillaries was rescued, at least partially, in double transgenic βB1-TGF-β1/βB1-Norrin mice.

Conclusion: Norrin and TGF-β signaling share a mutual inhibition of their pathways which appears to involve, at least partially Smad7 signaling.

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Reticular drusen associated with geographic atrophy in age-related macular degeneration

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Purpose: To characterize reticular drusen (RD) in patients with geographic atrophy (GA) secondary to age-related macular degeneration (AMD) in the multicenter, prospective natural history GAP-study using confocal scanning laser ophthalmoscopy (SLO) and spectral-domain optical coherence tomography (SD-OCT) imaging.

Methods: Three-field fundus autofluorescence (FAF, exc = 488, em 500 - 700 nm), near-infrared reflectance (NIR, λ = 830 nm), and blue reflectance (BR, λ = 488; Heidelberg Retina Angiograph/Spectralis, Heidelberg Engineering, Germany) images were recorded in 1104 eyes of 552 patients with GA (age 77.1 ± 7.7 years) in addition to color fundus photographs. Two independent readers evaluated baseline images for prevalence and topographic distribution of reticular drusen using a modified Early Treatment Diabetic Retinopathy Study (ETDRS) grid. In case of discrepancy, a third grader was asked to arbitrate. In a subset of 23 patients, simultaneous SD-OCT imaging was performed.

Results: RD were present in 326 of 552 (59.1%) patients in at least one eye and with at least one cSLO imaging modality (bilateral 234 [71.8%]). For each modality separately, prevalence of reticular drusen and kappa-statistics for interobserver reliability were as follows: IR 46.4% and 0.73, FAF 40.9% and 0.77, BR 21.3% and 0.63 (values for right eyes only, left eyes showed similar data). Systematic analysis of the topographic distribution using three-field FAF imaging (201 right eyes) demonstrated the presence of RD most frequently superior to the fovea (99.0%). In 85 (42.3%) right eyes, reticular drusen occurred nasal to the optic nerve head. SD-OCT imaging revealed alterations anterior to the RPE cell monolayer including focal deposits, hyperreflective migrating structures and regular wavy patterns.

Conclusions: RD represents a common phenotypic hallmark in eyes with GA secondary to AMD. In contrast to fundus photographs, RD are readily identified in various cSLO imaging modes. This may explain the high prevalence determined herein in contrast to previous reports based on fundus photographs. The corresponding morphological substrate in the outer neurosensory retina on high-resolution SD-OCT would reflect a disease process at the level of the photoreceptors rather than the biogenesis of the 'conventional' drusen phenotypes in the subretinal pigment epithelium space and in the inner aspects of Bruch's membrane.

Contractions of anterior human lens capsule epithelial cells

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Purpose: The single layer of epithelium is the first physical and biological barrier in the ocular lens. It has an important function in maintaining the lens internal milieu. To be able to perform this function its integrity is very important. Epithelial cells are tightly packed with very little intercellular space. Yet we have found that they often contract in response to various stimuli.

Methods: The capsules were stained with the fluorescent dye fura-2 and its fluorescence imaged upon excitation at 360 and 380 nm. The 360 nm excitation was used to monitor morphology of epithelial cells while the 360/380 ratio was used to monitor changes in $[Ca^{2+}]_i$. The preparations were stimulated with ACh as well as with jets of physiological saline alone and with direct mechanical stimuli.

Results: Although we used ACh we found that a mechanical stimulus alone is enough to induce a contraction. This contraction is both local and reversible. Contraction occurs wit<h all types of cataract studied (cortical, nuclear and combined cortical + nuclear). Contraction does not seem to be directly associated with the Ca-signalling. It exhibits a relatively fast response in the range of few tens of seconds.

Conclusions: This study provides the evidence that contractions of the anterior lens epithelial cells take place in significant proportion of human lens anterior capsule postoperative preparations in response to stimulation. Reversibility of the contraction suggests its physiological relevance as does the fast response time. The ability to contract seems to be an intrinsic property of the lens epithelial cells rather than a defect. The fact that the epithelial lens cell layer is a barrier protecting the inside of the lens suggests the importance of the cell contraction phenomena as a reaction to noxious and mechanical stimuli and may also influence the transport mechanisms of the lens. Although we currently do not have enough data to support it, the contribution of lens epithelial cells contractions to caractogenesis can also not be excluded.

Cell death mechanisms in two rat models for Retinitis Pigmentosa: S334ter-3 and P23H-1 rhodopsin mutations

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Purpose: In the rd1 animal model for Retinitis Pigmentosa (RP), retinal photoreceptor degeneration is caused by a deregulation of intracellular cGMP levels. To investigate a potential involvement of cGMP signaling to degenerative processes in rhodopsin mutants, we studied the levels of factors and enzymes related to cGMP metabolism in P23H-1 and S334ter-3 mutant rhodopsin rats, and corresponding wild-type retina.

Methods: Retinas of P23H-1, S334ter-3 (kindly provided by Dr. M. M. LaVail, University of California, San Francisco, CA) and CD rats were collected at different developmental ages (PN0 - PN30). Retinas were examined by immunohistochemistry on cryo- and paraffin- sections using specific antibodies against cGMP, PDE6beta and CREB.

Results: Using a well validated antibody directed against cGMP, we observed increased cGMP immunoreactivity in photoreceptor cell bodies, processes and segments compared to their age matched controls in both transgenic retinas, particularly in the S334ter-3 mutants. Early in postnatal development (PN12), cGMP immunostaining showed only a weak labelling in the ONL of wild type retinas and disappeared a few days later (after PN20). To determine if the improper cGMP accumulation follows PDE6β sorting alterations, we performed immunostaining in both rat models. PDE6β labelling was limited to the outer segments (OS) of photoreceptors in CD as well as in mutant rats, even though the OS length in S334ter-3 and P23H-1 is reduced as a consequence of the degeneration process. CREB expression was found in the cells localized in the OS, ONL, INL and GCL of CD retinas on all analyzed ages. On the other hand, in P23H-1 or S334ter-3 retinas, there was a clear reduction in the number of CREB stained cells especially in the ONL.

Conclusions: Our results suggest a critical role of cGMP and CREB in photoreceptor degeneration mechanisms in P23H-1 and S334ter-3 rats. Further experiments are required to establish the processes causing an accumulation of cGMP and its contribution to photoreceptor degeneration in rhodopsin transgenic rats.

Analysis of Crx-dependent Casz1 gene regulation using fluorescent reporter electroporation into explanted mouse retinas

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Cone-rod homeobox (Crx) is a photoreceptor-specific transcription factor, linked to retinal development and disease, but its in vivo targets are largely unknown. To address this point, we carried out genome-wide ChIP-Seq analysis of Crx target regions (CBRs). Amongst many others – castor zinc finger gene 1 (Casz1) was identified as a heavily Crx-bound gene. Since Casz1 is a zinc finger transcription factor that is highly expressed in neuronal tissues, we hypothesized that it may play an important role in retinal function. Therefore, our aim was to fully characterize the cis-regulatory elements of the Casz1 locus using electroporation of CBR-driven reporters in explanted living mouse retinas.

Individual CBRs were checked for phylogenetic conservation among various species. To determine the regulatory influence of distinct CBRs, they were cloned into a DsRed reporter vector system. The promoter CBR was cloned into a promoter-less vector (No-basal DsRed) whereas putative enhancer/silencer elements were cloned into a vector containing a bovine Rho promoter (Rho-basal DsRed). Furthermore, we established a series of combinatory clones, where non-promoter CBRs were cloned upstream of the promoter regulatory elements. The reporter constructs were then introduced into early postnatal (P0) mouse retinas using *ex vivo* electroporation. After 8 days of *in vitro* culture, fluorescence images were taken in both flatmounts and sections. First results of these analyses will be presented.

Identification of allelic expression differences in retinal expressed (disease) genes

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Purpose: Reduced penetrance and variability in disease expression with respect to onset, course, and severity is a well-documented feature in retinal dystrophies and hamper solid and reliable genotype/phenotype correlations as well as individual disease prognosis. Although the cause of this variability is largely unknown, it is commonly accepted that secondary genetic factors (modifier-genes) are key factors for the determination of the development, severity, and course of a disease. Based on known examples and high heritability of gene expression regulation, we hypothesize that *cis*-acting variants governing gene expression levels play a crucial role in phenotypic variation and disease penetrance in hereditary retinal disorders. The principle aim is the identification of such *cis*-acting gene variants and the determination of their impact on disease expression.

Methods: To achieve a heterozygous but genetic identical F1 generation with a high genetic variability, five different mouse inbred strains (C57BL/6, BALB/c, CAST/Ei, CBA/Ca & LP) were crossbred in all possible combinations.
15 different retinal disease genes were screened for heterozygous cSNPs applying PCR and sequencing. To determine allelic expression differences based on the identified cSNPs, we applied Pyrosequencing assays on RT-PCR amplified retinal cDNAs generated from retinal RNA. Results were calibrated for equimolar ratios by used genomic DNA as a control.

Results: Using the Pyrosequencing technology we detected an allelic imbalance (AI) in four out of 15 retinal disease genes. In all four genes *(Pde6c, Ush2a, Cerkl* and *Tlr4)* we identified the AI only on cDNA level. Screening of the Pde6c gene revealed a 116-bp insertion on cDNA level that results in a premature termination codon leading, due to the nonsense mediated mRNA decay, to a downregulation of the mutant transcript. For the remaining genes the cause of the AI has to be verified by determining the promoter regions and identify putative cis-acting variants applying reporter gene assays.

Conclusions: Until now, only in few cases *cis*-SNPs of retinal expressed genes could be identified which cause an allelic imbalance. Our results demonstrate that allele-specific differences in gene expression are common retinal expressed genes.

Differential expression of isoforms of the Usher syndrome scaffold protein harmonin in mouse and human photoreceptor cells

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Purpose: The human Usher syndrome (USH), the most frequent cause of inherited combined deaf-blindness, is divided into three clinical subtypes. USH1 is the most severe form characterized by profound inner ear defects, vestibular dysfunction and early onset of *retinitis pigmentosa*. The *USH1C* gene encodes the scaffold protein harmonin which is one of the major organizers of the USH protein interactome. Various alternative spliced transcripts are generated from the *USH1C* gene. Gene products are divided into a, b and c isoforms depending on their modular domain composition. To develop adequate and well-grounded therapeutic strategies, the knowledge of harmonin isoforms expressed in the retina is an essential prerequisite. So far, specific functions and expression profiles of the various isoforms are unknown. Here we investigate the expression of harmonin isoforms in murine and human retina.

Methods: Expression studies in murine and human retina by RT-PCR with isoform specific primer sets and Western blot analyses. Validation of protein-protein interactions via GST-pull down assays. Subcellular localization studies by correlative immunofluorescence and immunoelectron microscopy. Co-staining with anti-harmonin and cone-specific fluorescent peanutaglutinin.

Results: Expression studies revealed that harmonin isoforms a and b were expressed in murine retina. In addition, these isoforms were also recovered from mouse retina extracts in GST-pull down assays with harmonin PDZ domains, indicating heteromeric protein networks composed of different harmonin isoforms.
Results obtained by RT-PCR of human retina did not only confirm mouse data but also revealed previously unknown human harmonin isoforms. Western Blot analyses further support harmonin expression in human retina. Correlative microscopy analyses demonstrated subcellular localization of harmonin in outer segments of rod but not in outer segments of cone photoreceptor cells.

Conclusions: Our present study affirms doubted harmonin b isoform expression in the mouse retina. Furthermore, we demonstrate differential expression of harmonin isoforms in the

human retina for the first time. The obtained spatial characterization of harmonin indicates a specific scaffold function in human rod outer segments.

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Mutations in GCAP1 involved in hereditary cone dystrophy as a possible cause of phototransduction dysregulation

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Guanylate cyclase-activating protein 1 (GCAP1) is a Ca^{2+}-sensor expressed in rods and cones of the vertebrate retina. At its N-terminus GCAP1 is myristoylated and in this form it regulates the target enzyme, the photoreceptor guanylate cyclase (GC) in a Ca^{2+}-dependent manner. This GCAP/GC system is essential for phototransduction and light adaptation. Mutations in the gene encoding for GCAP1 are associated with cone dystrophies. One known and three so far unknown GCAP1-mutations within highly conserved EF-hand Ca^{2+}-binding sites were found to cause cone dystrophies. These are L151F (Jiang et al. Mol. Vis. 2005; 11:143-51) E89K, D100E and G159V (Kitiratschky et al. Hum. Mutat. 2009; 30: E782-96). Here we analyzed the GCAP1 mutants in their myristoylated and non-myristoylated forms with respect to the interaction with the GC, the Ca^{2+}-sensitivity of GC regulation, Ca^{2+}-induced conformational changes and the Ca^{2+}-binding properties. All GCAP-mutants were able to activate the GC with target affinities similar to the wildtype. However, Ca^{2+}-dependent GC-activation showed that all GCAP1-mutants activate the GC even at unphysiological high [Ca^{2+}]. Compared to the wildtype the shift in IC_{50}-values was 26- fold for the D100E-mutant, 16-fold for the G159V-mutant, 7-fold for the E89K-mutant and 34-fold for the L151F-mutant. With the non-myristoylated forms much higher shifts in IC_{50}-values, most notably with the L151F-mutant with a more than 100-fold higher IC_{50}-value, could be observed. Tryptophan fluorescence measurements in the absence of Mg^{2+} showed that Ca^{2+}-induced conformational changes for all GCAP-Mutants are altered. Using competition with a chromophoric chelator we found that compared to the myrGCAP1 wildtype all GCAP1-Mutants showed decreased Ca^{2+}-affinity (Dell´Orco et al. 2010). Our data indicate that the mutations probably decrease Ca^{2+}-affinity of the EF hand 3 or 4 leading to a permanent synthesis of cGMP under physiological conditions in cones and thereby trigger apoptotic pathways.

Jiang, L., Katz, B.J., Yang, Z., Zhao, Y., Faulkner, N., Hu, J., Baird, J., Baehr, W., Creel, D.J. and Zhang, K. (2005) Autosomal dominant cone dystrophy caused by a novel mutation in the GCAP1 gene (GUCA1A). *Mol Vis*, **11**, 143-151.
Kitiratschky, V.B., Behnen, P., Kellner, U., Heckenlively, J.R., Zrenner, E., Jagle, H., Kohl, S., Wissinger, B. and Koch, K.W. (2009) Mutations in the GUCA1A gene involved in hereditary cone dystrophies impair calcium-mediated regulation of guanylate cyclase. *Hum Mutat*, **30**, E782-796.
Dell'Orco, D., Behnen, P., Linse, S. and Koch, K.W. (2010) Calcium binding, structural stability and guanylate cyclase activation of GCAP1 variants associated with human cone dystrophy. *Cell Mol Life Sci*, **67**(6):973-84.

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Hereditary Retinal Disorders – From Patients towards Therapies

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Project: HOPE (Hereditary Retinal Disorders – From Patients towards Therapies; www.rd-hope.de) is one of a few disease-specific networks for rare diseases funded by the German Federal Ministry of Education and Research (BMBF) since April 2009. Research of the HOPE project focuses on hereditary retinal disorders (HRD) that represent a clinically and genetically heterogeneous group of ocular diseases with impaired light sensing and/or signal transmission within the neuroretina that result in loss of visual acuity, elevation of detection thresholds, constrictions or defects of the visual field and/or color vision defects. With about 30.000 people affected in Germany, HRD represent a clinically important group of diseases. There is currently no effective cure available.

Aim: HOPE aims at realizing new methodological and technical approaches for improving and further developing clinical and genetic diagnostics, and furthermore at promoting preclinical-stage identification, application strategies and validation of neuroprotective substances for the treatment of HRD. Due to the direct accessibility of the neuroretina to both, clinical investigations and surgical intervention, the involved researchers are confident of substantially improving and refining the diagnostics of HRD and of developing new therapeutic strategies close to human application.

Objectives: In committing to this goal, the HOPE consortium, structured in 7 subprojects, is focused on the following topics: 1) Development of new standards in clinical phenotyping, specifically implementation of refined electrophysiological methods and imaging technologies. 2) Evaluation of a resequencing DNA chip for the analysis of known HRD disease genes and its implementation into routine diagnostics. 3) Identification and validation of eQTLs as genetic modifiers for penetrance and disease expression in HRDs. 4) Identification and validation of bioactive, neuroprotective substances. 5) Further development of encapsulated cell technology (CellBeads®) as a tool for the in situ release of neuroprotective substances in models for HRD. 6) Development of an effective route of delivery for the newly identified therapeutic substances.

A new conditional photoreceptor degeneration and regeneration model in the adult zebrafish retina

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Purpose: Photoreceptor cell loss during adulthood is a major cause for blindness in humans. Studies of retinal degeneration and regeneration processes in regenerating model organisms are a powerful source of insight for retinopathy studies. Unlike mammals, zebrafish can regenerate their central nervous system (CNS) and retina. The wealth of experimental tools and the cone-dominated retina of zebrafish make it a good model for studies of human retinopathies.

Methods: We developed a new transgenic, conditional genetic lesion model in the Fgf pathway for analyzing photoreceptor degeneration and regeneration specifically in adult retinal tissue. We employed BrdU birthdating experiments, apoptosis assays and immunocytochemistry as well as transgenes driving lineage-specific GFP expression to study this model.

Results: Using conditional transgene expression, we specifically ablated photoreceptor cells, while other cells are largely unaffected. In our model, photoreceptors gradually die and have disappeared within one week after onset of transgene expression. Importantly, this conditional transgene-induced degeneration is completely reversible in the adult zebrafish retina, and regeneration can be traced from dividing progenitors into newly differentiated photoreceptors. Thus, a fast and profound regeneration response ultimately restores the layered retinal structure and vision by integrating new photoreceptor cells into the existing adult retina.

Conclusion: We established a new transgenic, conditional genetic lesion model for photoreceptors degeneration and regeneration for adult zebrafish retina. This model will be used to study the trophic mechanism of photoreceptor maintenance and the mechanisms that allow regeneration to occur in the adult vertebrate retina.

Fundus autofluorescence used for patient eligibility screening in a randomized interventional clinical trial on geographic atrophy secondary to age-related macular degeneration

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Purpose: To determine the frequency and specific causes of screening failures based on fundus autofluorescence (FAF) images in a multicenter, international, randomized clinical controlled trial (GATE-Study) for geographic atrophy (GA) in late stage age-related macular degeneration (AMD).

Methods: For screening of patient eligibility, confocal scanning laser ophthalmoscopy (cSLO) images were recorded with the Heidelberg Retina Angiograph/Spectralis (Heidelberg Engineering, Germany) and submitted by clinical sites according to the GATE-study protocol. Eligibility was determined by a central reading center from central near-infrared reflectance (NIR, $\lambda = 810$ nm), central blue reflectance (BR, $\lambda = 488$) and three-field fundus autofluorescence (FAF, exc. = 488, emission: 500-700 nm) images. Eligibility required: (1) high quality images, (2) GA secondary to AMD, (3) a well-demarcated lesion, (4) GA lesion total atrophy size ≤ 20 mm^2 (ca. 8 disc areas [DA]) and one single lesion of at least 1.25 mm^2 (0.5 DA), as quantified by a semi-automated image analysis software (RegionFinder, Heidelberg Engineering, Germany), and (5) increased FAF adjacent to an area of GA.

Results: From April to November 2009, screening failures based on FAF image analysis occurred in 270 of 1093 (24.7%) submitted subjects from 51 sites (mean submission: 21.4 subjects per site, range 1-54). Ineligibility of lesion size was the most common reason for screening failure (single lesion less than 1.25 mm^2 in 77 eyes (28.5%), lesion size exceeding > 20 mm^2 in 36 eyes (13.3%)), followed by insufficient image quality in 57 eyes (21.1%), no well-demarcated lesion in 47 eyes, often reflecting concurrent fibrosis/CNV (17.4%) and GA not secondary to AMD, (e.g. macular dystrophies) in 42 eyes (15.6%). Eleven (4.1%) were excluded because there was no abnormally increased FAF adjacent to GA. Screening failures of 24 large sites with more than 20 submissions varied considerably: 3.7 to 61.5%.

Conclusions: Screening for eligibility based on specific GA lesion criteria as determined by cSLO FAF imaging is possible in the context of a large-scale interventional clinical trial. With this screening, a clearly defined study population can be selected. Screening requires accurately quantifiable areas of GA, assumption of GA progression based on abnormal FAFsignal patterns

in the perilesional area, absence of poorly defined margins indicative of subretinal fibrosis and/or regressed CNV, GA due to late-stage dry AMD as differentiated from other causes such as hereditary macular dystrophies. The accurate identification of the study population will enable precise comparisons between control and treatment groups in interventional GA trials.

Retinal Stem Cells: isolation, maintenance and differentiation potential

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Purpose: Retinal degenerations (RD) are caused by the loss of retinal neurons like RGCs in glaucoma or photoreceptors in AMD or RP leading to progressive and irreversible loss of vision. One of the treatment strategies proposed for RD is to replace lost neurons with cells expanded in vitro, primed to specific neuronal fates that after transplantation would integrate into the retina and restore its visual function.

Materials and Methods: Retinal stem cell (RSC) cultures were generated by isolating retinal cells from eyes of postnatal day 0 (PN0) aGFP mice and expanded in medium supplemented with N2 and growth factors (EGF, FGF-2). RSCs were characterized by immunocytochemistry and RT-PCR by using antibodies against nestin, Pax6, ki67, b-III-tubulin, GFAP and primers for Rax, Chx10, Lhx2, Otx2, Pax6, Six3, Six6, Sox2, Notch1, Hes1 and Hes5, respectively.
For differentiation RSCs were subjected to a stepwise withdrawal of EGF and FGF-2 and replacement with 1% NCS. In parallel differentiation was carried out in the presence of the Notch signaling inhibitor DAPT. Differentiated cells were analyzed by immunocytochemistry using antibodies against b-III-tubulin, GFAP, MAP2, calbindin, calretinin, rhodopsin and recoverin. To investigate the differentiation potential of RSCs in vivo we performed transplantation experiments into the retinas of adult wt-mice. Perfusion-fixed experimental animals were analyzed by fluorescence/confocal microscopy following immunohistochemistry (b-III-tubulin, GFAP, MBP) on vibratome sections.

Results: Retinal cells can be expanded in vitro and maintain the potential to generate glial and neuronal cell types. However, RT-PCR analysis reveals that expanded RSCs loose expression of some genes that are characteristic for retinal progenitors, like Rax, Chx10, Six3, Six6, Otx2. Manipulation of Notch signaling by DAPT results in higher yield of b-III-tubulin-positive cells. RSCs injected into the retina.poorly integrate, forming layers on the vitreal side as well as in the subretinal space. Great majority of integrated cells develop into GFAP+ glial beside very few b-III-tubulin-positive neurons. Surprisingly, MBP immunoreactivity was detected in donor cells, indicating generation of myelinating oligodendrocytes.

Conclusion: RSCs cultures can be propagated as undifferentiated cells over several passages and maintain the ability to differentiate along the glial and neuronal lineage. Furthermore, the

proportion of neurons in vitro can be significantly increased by inhibiting the Notch pathway. However, expanded RSCs do not differentiate into true retinal neurons like e.g. photoreceptors, and lose expression of genes characteristic for retinal progenitors. Thus, it might be that used culture conditions lead to a loss of regional identity of RSCs. This interpretation is supported by the finding that transplanted RSCs have the capacity to differentiate into oligodendrocytes, a cell type that is not generated by RPCs in vivo.

Systems biochemistry approaches to vertebrate phototransduction: Toward a molecular understanding of disease

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Purpose: Phototransduction in vertebrates represents a paradigm of signalling pathways. The variety of protein-protein, protein-ion and protein-nucleotide interactions makes up an intricate network, which is finely regulated by activating-deactivating molecules and chemical modifications. The holistic systems properties of the network allow for typical adaptation mechanisms, which ultimately result in fine adjustments of sensitivity and electrical response of the photoreceptor cells to the broad range of light stimuli. An attempt to model the biochemical complexity underlying the signal transduction cascade in normal and altered conditions is presented.

Methods: We discuss a novel bottom-up strategy to study the phototransduction cascade in rod cells starting from the underlying biochemistry. The resulting network model can be simulated and the predicted dynamic behaviour directly compared with data from electrophysiological experiments performed on a wide range of illumination conditions. The advantage of applying procedures typical of systems theory to a well studied signalling pathway is also discussed.

Results: The network model was simulated and the results compared with experiments performed on dark and light-adapted rod cells, under a number of conditions in which illumination varied over five orders of magnitude. We show that such an approach provides a way to directly test model robustness and to suggest novel experiments that can potentially highlight the key interactions and components in the network. The model was also able to correctly predict photoresponses from *Rpe65*$^{-/-}$ animals, a model for Leber congenital amaurosis and vitamin A deprivation.

Conclusion: Applying the paradigm of systems biology to signal transduction in the retina by increasing the level of complexity in the description on a solid molecular background is expected to significantly contribute both to a better understanding of the biochemical processes and to the prediction of novel behaviours that emerge only at a systems level. This latter aspect is particularly promising as it opens new scenarios for the development of effective treatments of complex diseases.

GDNF-induced Mueller Glial-derived neuroprotective factors: Analysis of their survival effect in photoreceptors

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Purpose: Glial-Cell-Line-Derived Neurotrophic Factor (GDNF) is a powerful neuroprotective factor which enhances the survival of photoreceptors indirectly via Retinal Mueller Glial cells (RMG) (Hauck et al., 2006). The indirect survival action of GDNF goes through the stimulation of RMG, promoting the secretion of other neuroprotective factors which directly enhance photoreceptor survival. We have already identified and validated Osteopontin as a possible neuroprotective factor *in vitro* (Pro-Retina meeting, 2009). The purpose of this work is the functional validation of the survival effect of Osteopontin and some other possible neuroprotective factors in a mouse model of retinal degeneration.

Methods: Primary mouse RMG were isolated and treated with GDNF during 24 hours. The protein expression was tested in the lysates with a Mouse Angiogenic Array kit. Some of the upregulated angiogenic molecules (Fractalkine and Cyr61) were then tested for the *in vitro* neuroprotective effect in a photoreceptor survival bioassay. Osteopontin expression under GDNF stimulation was evaluated by immunohistochemistry and its survival effect was functionally assessed in C3H/HeH rd1 mouse retinal explants using a TUNEL assay.

Results: 18 angiogenic factors were differentially regulated by GDNF stimulation in mouse cultured RMG cell lysates. Osteopontin, Fraktalkine and Cyr61 were obtained as purified proteins and tested for activation of survival pathways in photoreceptors by western blot analysis and for survival promoting activity on primary photoreceptors *in vitro*. We found that Osteopontin, Fraktalkine and Cyr61 activate Akt survival pathway in photoreceptors at different time points and enhance survival of photoreceptors in culture. We also confirmed the upregulation of Osteopontin *in situ* under GDNF treatment, and the significant cell death reduction by Osteopontin treatment in rd1 mouse explanted retinas.

Conclusions: Among GDNF-induced molecules are novel candidate factors for neuroprotective activity. Currently we are testing some of these factors functionally and morphologically in different animal models of retinal degenerations for their therapeutic properties towards future clinical application.

Ultrastructural analysis of photoreceptor outer segments using rhoGFP transgenic mice

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Aim: Retinitis pigmentosa (RP) as one of the most common forms of inherited retinal degeneration is, beside age-related macular degeneration, diabetic retinopathy, or cone-rod dystrophy, characterised by a significant progressive loss of photoreceptor cells. Currently, there is no effective treatment available. Many current studies are focussing on a cell transplantational approach to replace degenerated photoreceptor cells using stem or progenitor cells. Up to now the successful transplantation and integration of cells into the outer nuclear layer (ONL) has been shown as well as some first hints that some of these cells manage to connect to bipolar cells and form inner and outer segments. Nevertheless, the key feature of every photoreceptor, to have a functional outer segment with properly aligned disc membrane staples has not been studied well so far. Here we show an ultra-structural analysis of outer segment integrity which might be useful for further transplantational studies.

Methods: For our studies we used rhodopsin-GFP fusion construct mice (rhoGFP). Transplanted cells were gained from postnatal day 4 rhoGFP retinas and transplanted into the sub-retinal space of wild-type mice using a Hamilton syringe. Further analysis was done by immunohistochemical staining of 200nm ultra-thin cryosections with anti-GFP antibody. For electron microscopy (EM) analysis a protein A gold staining was applied at labelled cryosections.

Results: We were able to detect the reporter protein on ultra-thin cryosections in very good quality. The anti-GFP antibody we used showed a very specific staining and nearly no background. After labelling with protein A gold particles and observation using an transmission electron microscope (TEM) we observed a very specific and well visible staining of outer segments with no background. Membrane disc staples were clearly visible in high contrast. Additionally we were able to rediscover transplanted rhoGFP cells. Their outer segments are specifically stained whereas the surrounding wild-type cells show no signal at all. The transplanted cells often start to develop membrane disc staples but we were not able to find a transplanted cell with properly developed and aligned discs. Surprisingly, a lot of cells which didn't manage to integrate and staying in the subretinal space seem to be successful in forming outer segments with intact and well organised disc membrane staples.

Conclusion: We could show that our method is very suitable for detailed examination of outer segments of transplanted photoreceptor cells which might be very useful for further transplantational studies.

Blue light-induced microglial activation and transcriptomic changes in the mouse retina: Evidence for signaling mechanisms different from white light

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Purpose: Microglia and macrophages are important components of the ocular immune system and may also contribute to age related macular degeneration (AMD). Exposure to blue light induces oxidative protein modifications similar to those observed in Drusen and elicits AMD-related immune responses. To further clarifiy the underlying cellular events, we studied microglial activation and monitored global transcriptomic changes in blue light-induced retinal lesions.

Materials and Methods: Transgenic MacGreen mice, which express GFP in retinal microglia, were exposed to 410 ± 10 nm blue light. At different time intervals after treatment, eyes were prepared for immunofluorescence microscopy, qRT-PCR and microarray analysis.

Results: Retinal whole mounts and cross sections showed that GFP labelled amoeboid microglia rapidly migrated towards the central retinal lesion. Prominent transcriptomic changes occurred in the retina after 12h, peaked at 24h and declined at 72h. We identified more than 50 differentially expressed genes related to pro-inflammatory microglial activation, apoptosis and cell damage as well as 10 previously uncharacterized transcripts. Interestingly, different endogenous rescue pathways were activated, as demonstrated by Lif/Jak/STAT signaling and up-regulation of anti-apoptotic transcripts (Tmbim1), regeneration genes (Sprr1), and microglia secreted dampening molecules (Crispld2). We could not detect expression of c-jun, c-fos, S100 genes or crystallins, as previously reported for retinal responses to white light damage.

Conclusion: Our results demonstrate activation of different transcriptional phenotypes of retinal microglia in blue-light induced lesions. Moreover, the molecular components of both apoptosis and survival signaling are distinctly different from those previously reported for acute and chronic white light lesions.

Spectral domain OCT of increased fundus autofluorescence in the perilesional zone of geographic atrophy due to AMD

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Purpose: To determine microstructural correlates on spectral-domain optical coherence tomography (SD-OCT) of areas with increased fundus autofluorescence (FAF) in the perilesional zone of geographic atrophy (GA) due to age-related macular degeneration (AMD).

Methods: In a prospective longitudinal study (FAM study, NCT00393692), 120 eyes of 67 patients (mean age 76.1±8.6 years) with uni- and multifocal GA due to AMD were examined by simultaneous confocal scanning laser ophthalmoscopy and high-resolution SD-OCT (Spectralis HRA+OCT, Heidelberg Engineering). FAF patterns with dependent progression rates were classified as previously described (Am J Ophthalmol. 2007;143:463-72). Corresponding microstructural changes of the outer retinal layers were evaluated in SD-OCT scans.

Results: FAF patterns were classified into "focal" (7 eyes, 5.8%), "banded" (14 eyes, 11.6%), "patchy" (6 eyes, 5%), "branching" and "reticular" (33 eyes, 27.5%), "fine-granular" (37 eyes, 30.8%), "GPS" (4 eyes, 3.3%), and "trickling" (18 eyes, 15%); one eye displayed no abnormal FAF. SD-OCT revealed clearly definable outer retinal layers without marked alterations in areas with a normal FAF signal. Circumscribed lesions with intensive increased FAF in eyes with "focal", "branching", "reticular" and "GPS" FAF patterns, correlated with hyperreflective material that was located between the retinal pigment epithelium/Bruchs membrane- (RPE/BM) and outer nuclear layer-level. Areas with increased FAF at the GA border in eyes with the "banded" pattern and in a more widespread area in eyes with the "fine-granular" and "trickling" FAF pattern correlated with a thickened RPE/BM layer,and frequently with apical extension. In areas of increased FAF, the integrity of the photoreceptor inner segment/outer segment layer was severely disturbed. A splitting of the RPE/BM layer in the perilesional zone through the border was most obvious in eyes displaying the "trickling" FAF pattern with the highest progression rates.

Conclusions: In areas with increased FAF outside areas of GA, SD-OCT imaging reveals marked morphological abnormalities not only of the RPE cell layer but also of outer layers of the neurosensory retina. Pronounced photoreceptor layer alterations may indicate a pathophysiological role early in the disease process in areas of incipient atrophy identified by FAF imaging. In longitudinal studies, combined FAF and SD-OCT imaging may add to the spectrum of biomarkers for lesion growth.

Imputation and analysis of a genome-wide association study of age-related macular degeneration (AMD)

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Introduction: Age-related macular degeneration (AMD) is a complex disorder of the retina/choroid and the most common cause of legal blindness in the industrialized countries. Two major susceptibility loci have been identified including *CFH* on 1q31 and *ARMS2/HTRA1* on 10q26. Furthermore, two additional complement genes (*C3* and *C2/CFB*) have also been shown to play a role in AMD pathogenesis. Other susceptibility genes with moderate to minor contributions to AMD risk are likely but difficult to detect in genome-wide association studies (GWAS). In particular, polymorphic marker density and statistical power are two major hurdles in these efforts. Imputation methods have recently been introduced which facilitate (1) a reliable increase of marker density without additional genotyping and (2) a meta-analysis of GWAS even in cases where different SNP panels were used for genotyping. The aim of our study was to perform an imputation of an available GWAS data set and to initially analyse the resulting genotypes.

Methods: The GWAS consisted of 335 late-stage AMD patients which were genotyped with the Affymetrix 250K *Sty*I array and of 1,636 population-based controls (KORA study, Augsburg) genotyped with the Affymetrix 500k array set (250k *Sty*I and 250k *Nsp*I). Imputation was performed using the "BEAGLE Genetic Analysis Software Package" which employes the phased haplotype data from 90 unrelated, Caucasian individuals (HapMap CEU, Release 22) as reference sample to impute the genotypes of up to 2.5 Mio SNPs. The association tests were performed using "SNPTEST" which incorporates the genotype probabilities and takes account of the imputation uncertainty.

Results: Before imputation we tested 174k SNPs meeting our quality criteria for their association with AMD. This revealed four SNPs localized within the two known susceptibility loci at 1q31 and at 10q26 which obtained genome-wide significance. After imputation with a total runtime of approximately 20 days, an eight fold increase in the number of analyzable SNPs was obtained (N ≈ 1.4 Mio). As before imputation, statistically significant association signals were only found at loci 1q31 and 10q26. Notably, their association signals markedly increased

in number and strength. An increase of the overall background noise was not noticed indicating the reliability of the imputation.

Conclusions: As imputation methods are based on phased haplotypes, the testing of imputed SNPs represents a method to indirectly assess haplotypic associations which otherwise would be computationally demanding and error-prone on a genome-wide level. Nevertheless, no additional frequent susceptibility locus for AMD was identified indicating that not marker density alone, but statistical power is indispensable for their detection. Further progress in imputation methods, e.g. the completion of the 1,000 genomes project, will enhance the reliable estimation of even lower frequent SNPs and will have the potential to search for moderate to minor risk factors in complex diseases.

PTC124 therapy for a nonsense mutation causing Usher syndrome type 1C

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Purpose: The human Usher syndrome (USH) is the most frequent cause of inherited combined deaf-blindness. It is clinically and genetically heterogeneous, assigned to three clinical USH types of which the most severe type is USH1. So far no effective treatment for the ophthalmic component of USH exists. PTC124 is a promising compound for translational read-through of nonsense mutations leading to a premature termination stop, currently gauged in clinical phase II for nonsense mutation in non-ocular diseases. Here we investigated the potential of PTC124 as a treatment option for patients carrying nonsense mutations in the *USH1C* gene (p.R31X) causing the USH1 disease.

Methods: Read-through of PTC124 was validated in cell culture and retinal explants. Restoration of the USH1C protein harmonin function was tested in GST-pull down analyses and by co-stainings. Biocompatibility was determined in murine and human organotypic retinal cultures by TUNEL-assays. In all assays the application of the aminoglycoside gentamicin were used as controls and compared with PTC124 treatments.

Results: PTC124 induced read-through of the *USH1C* p.R31X mutation was observed in HEK293T cells. The recovered expression restored harmonin´s scaffolding function and F-actin bundling activity. Treatment of PTC124 resulted in a read-through in p.R31X transfected retinal explants. In contrast to gentamicin, PTC124 treatment did not induce cell death in murine and human retinas indicating a high retinal compatibility of PTC124.

Conclusion: PTC124 mediated read-through of the p.R31X nonsense mutation in *USH1C* effectively restored full-length protein expression and recovered the scaffolding function of the USH1C protein harmonin. PTC124´s high retinal compatibility combined with its transcriptional read-through efficacy emphasize the high potential of PTC124 as a therapeutic agent for nonsense mutations in *USH1C* and other ocular and non-ocular diseases.

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Functional characterization of PDE6C mutations linked to autosomal recessive achromatopsia

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Purpose: Achromatopsia is a rare congenital and stationary disorder with an autosomal recessive mode of inheritance. Rod monochromacy is characterized by reduced visual acuity, pendular nystagmus, photophobia, reduced or complete loss of color discrimination. To date four genes have been identified for achromatopsia that are all functional components of the vertebrate cone photoreceptor visual transduction cascade. We have examined six missense mutations within the PDE6C gene, which encodes the alpha´- subunit of the cone-specific phosphodiesterase, to clarify the functional consequences.

Methods: Expression constructs applying the pFastBac HTb vector (Invitrogen, Carlsbad, USA) for human PDE6C/PDE5-chimeras were cloned and achromatopsia associated mutations introduced by an *in vitro* mutagenesis strategy. Proteins were expressed in the baculovirus-Sf9-expression system and monitored by SDS-PAGE, Western blotting and Coomassie staining. The proteins were purified using affinity chromatography on a His-bind resin (Novagen, Darmstadt, Germany). The functionality of wild type and mutant recombinant proteins was analyzed by a series of assays including gel filtration, enzymatic activity, and inhibition by Zaprinast, IBMX and the inhibitory gamma-subunit.

Results: All mutations result in considerable to total loss of PDE6C-specific enzymatic activity and/or altered substrate binding, as well as altered binding and inhibition by Zaprinast, IBMX and the inhibitory gamma-subunit.

Conclusion: Only few mutations in *PDE6C* have been observed in patients with autosomal recessive achromatopsia, including six missense mutations. We provide evidence that all analyzed missense mutations are true pathogenic mutations.

Partial colocalization of retinoschisin (RS1) and Na^+/K^+-ATPase subunits in membrane rafts

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Purpose: Previous studies have shown that RS1 binds to the Na^+/K^+-ATPase within the retina. In other cell types like cardiac myocytes the Na^+/K^+-ATPase is located in detergent resistant membranes (DRMs), which represent rafts floating on the cell surface. These membrane domains can serve as platforms for receptor-ligand interactions and intracellular signalling. We hypothesized that RS1 binds to the β2-subunit of the α3β2 Na^+/K^+-ATPase in retinal rafts and thereby regulates signalling functions. To demonstrate the colocalisation of RS1 and the raft Na^+/K^+-ATPase, DRMs from WERI-RB1 cells and mouse retinas were analyzed.

Methods: To isolate DRMs from WERI-RB1 cells and mouse retinas, cells were pelleted and lysed after treatment with 2% Triton-X 100 at 4°C. A discontinuous density gradient centrifugation was then performed with OptiPrep®. After 4 hours of centrifugation at 100.000xg, six fractions were collected from the top to the bottom. The proteins were precipitated by methanol/chloroform before resuspension in buffer containing 1% SDS. The samples were then analyzed by Western blotting using antibodies against the β2 subunit of the Na^+/K^+-ATPase, RS1 and flotillin as a DRM-marker.

Results: We could successfully implement raft isolation from WERI cells and mouse retinas. DRMs were floating up in the low density fractions as demonstrated by strong staining with the marker flotillin. Staining for the β2 subunit of the Na^+/K^+-ATPase showed also high intensities in raft fractions in WERI cells. In retinal samples, staining for the β2 subunit of the Na^+/K^+-ATPase was strong in the raft fraction, but was also present in high density fractions. We could also show that RS1 was partially present in DRMs of WERI cells and retinal cells.

Conclusion: Our experiments showed that the α3β2 subunits of Na+/K+-ATPase and RS1 partially colocalized in retinal rafts. This distribution of RS1 implicates that it has a dual function. On the one hand, RS1 may serve as a secreted adhesion molecule and on the other hand it may trigger intracellular signalling by interaction with the Na^+/K^+-ATPase in rafts as signalling platforms.

Characterization of a transgenic ARMS2 mouse line – a potential model for age-related macular degeneration (AMD)

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Introduction: AMD is a multifactorial disease of the central retina and a leading cause of irreversible vision loss in developed countries. A total of 15 genetic variants in chromosomal region 10q26 have been strongly associated with the disease and are located within a 23.3 kb region of strong linkage disequilibrium (LD) (1). These polymorphisms center over two nearby genes: ARMS2 (age-related maculopathy susceptibility 2) and HTRA1 (HtrA serine peptidase 1). To determine the causative mutation for increased AMD susceptibility, functional analyses are required. Here, we focus our analyses on ARMS2, an evolutionarily recent gene within the primate lineage.

Methods: Three independent ARMS2 transgenic mouse lines were generated. An ARMS2 genomic construct was cloned under the control of the murine rhodopsin promoter for ensuring heterologous ARMS2 expression in mice photoreceptor cells. The construct was injected into CB6 zygotes by Polygene AG (Rümlang, Switzerland) resulting in three independent ARMS2 transgenic mouse lines. To quantify the relative copy number of ARMS2 in the three transgenic mouse lines, quantitative real-time (qRT)-PCR was performed with genomic DNA. ARMS2 expression in the retina of transgenic animals was investigated by immunohistochemistry (IHC), Western blot analysis, and reverse transcription (RT)-PCR. Phenotypic characteristics of transgenic mice were determined by semi-thin eye sections.

Results: qRT-PCR revealed differences in the copy number of the integrated ARMS2 transgene among the different mouse lines. This approach also enabled us to discriminate between consanguineous offspring carrying the transgene on one or on both homologous chromosomes. One of the ARMS2 transgenic mouse lines exhibited an X-chromosomal inheritance pattern. No prominent phenotype was visible in semi-thin eye sections of ARMS2 transgenic mice compared to wildtype animals. The presence of the ARMS2 transcript in the retinae of ARMS2 transgenic mice was detected only sporadically despite the genomic presence of several transgene copies. Similarly, in these mice the ARMS2 protein could not be detected in Western blot analysis and IHC staining.

Conclusions: The three ARMS2 mouse lines differ in their relative amount of integrated transgene copy numbers and chromosomal integration sites. Nevertheless they share a similar sporadic ARMS2 expression among the offspring making further studies difficult. Moreover heterologous ARMS2 expression in murine photoreceptors does not lead to a prominent alteration in the morphological phenotype as revealed by semi-thin sections.

Literature: 1) Fritsche et al., 2008, Nat. Genet., 40:892-6

Degenerating Photoreceptors in the RCS Rat can be rescued by an AAV2/4-RPE65-Mertk Vector

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Purpose: The RCS rat with its mutation in the Mertk gene and therefore the loss of phagocytic function of the retinal pigment epithelium (RPE) is an established model for *retinitis pigmentosa*. We checked whether photoreceptors can be rescued from degeneration by the introduction of an intact copy of the rat Mertk gene by an adeno-associated viral vector.

Methods: We used an AAV2/4-RPE65-Mertk vector with a high specificity for RPE cells and a high effectiveness of Mertk expression. A solution of the vector was injected subretinally into the eyes of 13 20-day-old RCS rats. As a control, PBS alone was injected in three rats, and sham surgery or no treatment was performed in 14 rats. After one or two months, protective effects were evaluated by checking retinal function by electroretinography (ERG), inspection of the eyes by optical coherence tomography (OCT) as well as histological and ultrastructural evaluation of sections of the eyes.

Results: Enhanced ERG amplitudes compared to the control eyes could be recorded in 8 out of 13 eyes injected with the AAV Mertk vector one month after the injection of the vector, and in 7 out of 10 eyes two months after the injection. Histological inspection of the eyes revealed a well-preserved photoreceptor layer, though restricted to a part of the eye, in 8 out of 12 eyes one month after the injection of the vector, and in 6 out of 8 eyes two months after the injection, whereas normal degeneration was found in 11 out of 13 eyes injected with PBS. Enhanced survival of photoreceptors was found in particular in those eyes where increased ERG amplitudes had been measured before. An increased thickness of the retina was also found by OCT, simultaneously with a decreased autofluorescence in the rescued area. Moreover, phagosomes could be detected in the RPE by electron microscopy two months after an injection of the vector.

Conclusions: We found that degenerating photoreceptors in the RCS rat can be rescued and their function can be preserved by a subretinal injection of an AAV Mertk vector. The results encourage the search for a gene therapy for *retinitis pigmentosa*.

Interaction of retinoschisin with the Na^+/K^+-ATPase β_2 Subunit

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Purpose: X-linked Retinoschisis (XLRS) is a common cause of juvenile macular degeneration in males caused by mutations in the *RS1* gene. The *RS1* gene encodes retinoschisin, an extracellular adhesion protein that is expressed and secreted from photoreceptor and bipolar cells. It associates with the extracellular surface of these cells where it is assumed to serve in the maintenance of the cellular organization of the retina and the photoreceptor-bipolar synaptic structure. In a recent study an association of retinoschisin with the Na^+/K^+-ATPase was shown (Molday et al. JBC 282:32792-801, 2007) although the exact nature of this interaction remains to be determined.

Methods: To further investigate the interaction of retinoschisin with the Na^+/K^+-ATPase and to identify the ATPase subunit interacting with retinoschisin, co-immunoprecipitation was performed. In addition, we resorted to ex-vivo binding-assay studies with retinae of *Rs1h*-knock out mice, *Atp1b2*-deficient mice and double knock out animals ($Rs1h^{(-/Y)}$ / $Atp1b2^{(-/-)}$). Via immunohistochemical stainings of retinoschisin and ATPase subunits α_3 and β_2 in wildtype and $Rs1h^{(-/Y)}$ mice as well as Na^+/K^+-ATPase $\beta_2{}^{(-/-)}$ retinae we investigated co-localization and potential pathological events at different stages of development.

Results: Our data suggest a direct interaction of retinoschisin with the Na^+/K^+-ATPase β_2 subunit. *Atp1b2*-deficient mice show a markedly reduced protein level of retinoschisin significantly decreasing in its amount in postnatal weeks PN1 and PN2. Furthermore, in an ex-vivo binding assay with tissue lacking the Na^+/K^+-ATPase β_2 subunit the retina-specific binding of retinoschisin is absent. In contrast, in *Rs1h*-knock out mouse the amount and localisation of the ATPase β2 subunit within the retina is not altered.

Conclusion: The binding of retinoschisin to the β_2 subunit of the Na^+/K^+-ATPase appears to anchor retinoschisin at the cell surfaces of photoreceptor and bipolar cells. Further investigation into downstream gene and protein networks associated with retinoschisin binding to Na+/K+-ATPase β_2 will be essential to gain insight into the functional role of retinoschisin in the healthy retina but also the pathological process underlying XLRS.

The Crx target Samd7 is specifically expressed in the nucleus of developing and adult mouse photoreceptors

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Purpose: The transcription factor Crx (Cone-rod homeobox) is a master regulator of photoreceptor gene expression. For a genome-wide identification of Crx-bound cis-regulatory regions, chromatin-immunoprecipitation (ChIP)-sequencing was used. The highest binding affinity of Crx was confined to the Samd7 gene, which encodes a novel sterile alpha motif domain-containing protein. Our aim was to characterize the expression, localization and function of Samd7.

Methods: qRT-PCR was used to analyze the expression of Samd7 in eight different developmental stages beween postnatal days P0 and P28. The subcellular localization of Samd7 was studied in transiently transfected HEK293 cells by Western blotting and immunocytochemistry. *In situ* visualization of Samd7 in retinal sections was performed by immunofluorescence microscopy.

Results: qRT-PCR showed that Samd7 mRNA levels steadily increased between P0 and P5 and high amounts of Samd7 transcripts were present until adulthood. Initial immunocytochemistry experiments of His-tagged Samd7 showed a cytoplasmic localization in HEK293 cells using an anti-Samd7 antibody. However, Western blot analysis of the same cells revealed a nuclear localization. To clarify this discrepancy, we expressed Samd7 without a tag and immunochemistry then showed a clear nuclear staining. In agreement with these data, immunohistochemistry on retinal sections at P7 and P21 localized Samd7 in the outer nuclear layer.

Conclusion: Samd7 is a Crx-regulated gene that is expressed at high levels in developing and adult photoreceptors. *In vitro* overexpression and *in situ* staining indicate nuclear localization of Samd7. It is tempting to speculate that this protein is an important nuclear factor in photoreceptors.

Towards a stem cell-based intraocular delivery system for therapeutic gene products

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Objective: Continuous and long-lasting intraocular delivery of therapeutic gene products, including neuroprotective factors for photoreceptor cells, might be achieved by transplantation of genetically engineered stem cells. With the aim to develop such a stem cell-based intraocular delivery system, we have generated a panel of lentiviral vectors to express various neurotrophic factors, either single or in combinations, in murine neural stem cells (NSCs) or mesenchymal stem cells (MSCs).

Methods: To genetically modify stem cells, we have generated bicistronic lentiviral vectors consisting of a chicken ß-actin (CAG) promoter, a "gene of interest" (here: glial cell line-derived neurotrophic factor (GDNF)), an internal ribosome entry site (IRES) and different combinations of reporter/resistance fusion genes downstream of the IRES. Lentiviral particles were pseudotyped with the envelope of the ecotropic murine leukaemia virus, and used to transduce murine NSCs or MSCs by spinoculation. To express two or more transgenes, cells were transduced with several vectors each encoding a different combination of reporter/resistance fusion genes. Positive cells were selected by application of antibiotics or FACS, and analyzed for expression of transgenes by immunocytochemistry and immunoblot analysis. Furthermore, modified NSCs and MSCs were used for first intraretinal and intravitreal transplantations into *rd1* and wild-type mice, respectively.

Results: Spinoculation of NSCs and MSCs and subsequent selection of positive cells allowed rapid derivation of stem cell cultures with expression of reporter genes in apparently every cell. Immunocytochemistry and immunoblot analysis confirmed expression of GDNF in both stem cell types, and secretion of this neurotrophic factor into the culture supernatant. Co-expression of transgenes was also successfully achieved, as indicated by the simultaneous expression of two or more reporter genes. Intraretinally grafted engineered NSCs and intravitreally grafted engineered MSCs survived and expressed the transgenes for at least one month in host retinas, the longest post-transplantation interval analyzed.

Conclusions: We have generated a panel of lentiviral vectors that allow rapid derivation of genetically engineered murine NSC or MSC cultures. Transplantations of modified stem cells with expression of one or more neuroprotective factors into mouse models of retinal dystrophies will reveal the therapeutic potential of this stem cell-based delivery system.

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Loss of amacrines not photoreceptors stimulates regeneration of amacrines

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Background and aims: Retinal regeneration is well established in non-mammalian vertebrates. Recent reports have found that to a limited extent similar processes can be induced in adult rodent retina. Here we tested for neuronal regeneration after light damage of photoreceptors in comparison to neurotoxic damage of retinal interneurons and ganglion cells of adult murine retina in vivo.

Methods: We examined regeneration in adult mice. To address this question in vivo we damaged adult mouse retina by intraocular injection of neurotoxin NMDA (loss of amacrine and retinal ganglion cells) or by light damage (loss of photoreceptor cells). Two days later we subsequently injected growths factors. Several growths factors were tested and animals were sacrificed at various time points. Retinas were analyzed using immunostaining.

Results: Here we provide further evidence that Müller glia are the source for neural regeneration and we show that loss of retinal ganglion and amacrine cells, but not photoreceptors increases the number of regenerating amacrines. Müller glia express GFAP upon retinal injury. Using a genetic lineage trace we stimulated a population of GFAP expressing glia to proliferate and regenerate amacrines cells upon neurotoxic damage. Exposure to bright light leads to photoreceptor damage and subsequent injection of growth factors does not increase the number of regenerated GAD67-GFP neurons (amacrines) compared to growth factor injections alone. In contrast, NMDA induced neurotoxic injury with subsequent growth factor stimulation led to a significant higher number of regenerated GAD67-GFP neurons. None of the treatments we tested so far after retinal light or NMDA damage led to regeneration of photoreceptors (Nrl-GFP), bipolar (mGluR6-GFP) or retinal ganglion cells (Thy1-CFP) in adult mice in vivo.

Conclusion: Our results suggest that a restricted number of Müller glia may regenerate amacrines in living adult mice; this process is damage dependent in that loss of amacrines but not loss of photoreceptors promotes amacrine regeneration. We conclude that mechanisms to signal amacrine loss as a component of a retinal regenerative program exist and are in place while others are limited or absent.

The novel Activated Microglia/Macrophage WAP Domain Protein, AMWAP, is induced during Microglial Activation and acts as a negative Regulator of Pro-Inflammatory Response

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Purpose: Chronic microglia activation is a common phenomenon in neurodegenerative disorders including retinal dystrophies. To identify genes involved in this process, we used DNA-microarray analysis of retinal microglia from wild-type and retinoschisin-deficient *(Rs1h$^{-/Y}$)* mice, a model of inherited retinal degeneration. Thereby we discovered a microglia-restricted whey acidic protein that was highly expressed in activated retinal microglia. As other WAP domain proteins play a role in immune homeostasis, our aim was to characterize the function of AMWAP with a special focus on inflammatory processes.

Methods: A recombinant AMWAP-GFP fusion protein was over-expressed in BV-2 microglia to detect its subcellular localization. Over-expression and knock-down of AMWAP were carried out to determine inflammatory marker transcript levels by qRT-PCR. Stimulation of different immune-related receptors was performed to clarify mechanisms of AMWAP activation. Trypsin-protection assays and anti-bacterial assays were used to study protease-inhibitory and anti-bacterial effects of AMWAP.

Results: Expression of an AMWAP-GFP fusion-protein in microglia decreased transcript levels of pro-inflammatory cytokines and chemokines, whereas anti-inflammatory arginase 1 was induced. Conversely, knock-down of endogenous AMWAP augmented pro-inflammatory gene expression. AMWAP transcripts were strongly induced upon stimulation of microglia with LPS, Inf-gamma, CpG oligonucleotides and palmitoylated lipopeptides. AMWAP expressing cells also showed improved adhesion in a trypsin-protection assay indicating anti-serine protease activity. Moreover, recombinant AMWAP exhibited potent growth inhibitory activity against *E. coli, P. aeruginosa and B. subtilis at micromolar* concentrations.

Conclusions: We hypothesize that AMWAP is a counter-regulator of pro-inflammatory microglia activation in the degenerating retina. Thus, this protein may be beneficial in converting neurotoxic microglia into a neuroprotective phenotype.

Modifications in retinal neurons and synaptic connectivity during photoreceptor degeneration in two Rhodopsin mutant transgenic rats

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Purpose: to evaluate changes in cone photoreceptors, horizontal cells and synaptic markers during retinal degeneration.

Methods: Retinas from heterozygous P23H-1, S334ter-3 and CD rats were collected at various postnatal ages (PN0 - PN30) and were examined by immunohistochemistry using specific antibodies against M-opsin, calbindin, connexin36, mGluR6 and bassoon.

Results: At PN30 almost all cones survive after the initial degeneration of the rods in both mutant models. During this time, cones first lose their outer segments and then they partially retract (P23H-1) or lose (S334ter-3) their axon and synaptic pedicle. However, they retain their laminar position, continue expressing opsin and show a polarized morphology. At this age, the gap junctions in the pedicles of adjacent cones stained with connexin36 antibody were clearly reduced in P23H-1 and no longer expressed in the S334ter-3 retina. mGluR6 (post-synaptic marker) immunostaining localized to the dendritic tips of ON bipolar cells was reduced in both mutants. A similar decline in the number of photoreceptor terminals immunostained with bassoon (pre-synaptic marker) was also found. No alteration in the IPL staining was observed. Horizontal cells immunostained with calbindin showed normal sized somata but the density of their processes in the OPL was reduced. We found soma displacement into the remaining ONL and we observed sprouting of processes from horizontal cells in P23H-1 and S334ter-3, oriented radially towards the INL. In the case of S334ter, these processes were longer and they end at the border with the IPL.

Conclusions: During the course of photoreceptor degeneration in rhodopsin transgenic rats with two different mutations, there are progressive degenerative changes that are very similar to described in other animal models of retinitis pigmentosa. We believe that several alterations in second-order neurons have common mechanisms independent of the primary cause of the photoreceptor degeneration and should be carefully taken into consideration while working towards the development of neuroprotective treatments.

Blue light collagen cross linking to treat progressive myopia

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The aim of this study was to develop an effective and applicable method to treat progressive myopia. Myopia is the most common eye disorder of the world with an incidence of 20-30 % in Western Europe and up to 90 % in Asian regions, respectively. About 50 % of people with myopic eyes suffer from a so called progressive (axial) myopia characterized by an excessive eye growth and severe pathological changes of the retina. The biomechanical weakness of the sclera causes an excessive intraocular pressure-driven axial elongation of the eye. The idea is to use Riboflavin/blue light collagen cross linking to increase the scleral stiffness and thereby, to stop eye growth.
The sclera/eye of adult and young postnatal rabbits was treated with Riboflavin/blue light. A series of experiments reveal the exact parameters for the adequate laser intensity, irradiation time, riboflavin concentration and soaking time. The animals were monitored ophthalmologically over a period of 3 weeks and subsequently, the eyes were isolated and examined by means of biophysical methods, histology, immunohistochemistry and electron microscopy.
Young animals treated with a suitable laser energy of about 10 mW/cm^2 displayed a clear reduction of their eye growth as compared to the fellow control eyes. Immunohistochemical examinations showed no neurodegenerative side effects or signs of glial reactivity using an intensity lower than 200 mW/cm^2. In adult rabbit eyes the critical treatment intensity was 400 mW/cm^2. Electron microscopy reveals a decrease of mean collagen fibril diameters after cross linking, especially in the outer sclera. Biomechanical measurements of isolated scleras from adult rabbits revealed increased stiffness/rigidity after collagen cross linking.

Molecular dissection of ARMS2 secretion

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Purpose: ARMS2 is one of the three genes in close vicinity on chromosomal region 10q26 strongly associated with age-related macular degeneration (AMD). Recent data have indicated that ARMS2 is causally related to the deterioration of vision in individuals carrying the risk haplotype(s). We showed that ARMS2 interacts with extracellular matrix proteins in the choroid, thereby connecting proteins already implicated in various (familiar and sporadic) macular dystrophies. Furthermore, its deposition in the intercapillary pillars corresponds to the principal sites of drusen formation. To gain an understanding of the mechanism responsible for the extracellular targeting of ARMS2, this alternative secretory route was further dissected.

Methods: Cassette mutagenesis was used to generate a series of plasmids coding for mutated ARMS2, where single amino acids in the C-terminal region of the protein were substituted with alanine (alanine screen). The localizations of the mutated ARMS2 proteins in transfected cells were revealed by immunocytochemistry using our own ARMS2-specific monoclonal antibodies. A blast search within the human protein database was undertaken, in order to identify further proteins possessing the same putative targeting signal.

Results: Within the last eight amino acids (-SIIHTAAR*) encoded by the second exon of ARMS2, the presence of the two neighboring isoleucines is indispensable for proper targeting. The substitution of the other amino acids did not result in subcellular localization distinguishable from the wild-type situation. The same C-terminal double isoleucine motif was found in further 64 human proteins. This subset of proteins also includes TIMP-3, a protein implicated in Sorsby's fundus dystrophy.

Conclusions: The secretion of ARMS2 suggests an alternative secretory targeting of the protein, depending on a C-terminal signal sequence and lacking a classical, N-terminal signal peptide. Tissue inhibitor of metalloproteinases 3 (TIMP-3), a protein implicated in Sorsby's fundus dystrophy, shares this C-terminal motif, and may also use it to commit to secretion. Because ARMS2 localizes primarily at sites corresponding to drusen deposition, this secretory mechanism may play an important role in the pathogenesis of AMD.

Deciphering the cis-regulatory elements of the RS1 gene – optimizing the vector design for therapeutic gene delivery

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Purpose: X-linked retinoschisis (XLRS) is an orphan disease characterized by juvenile macular degeneration. It is caused by mutations in the RS1 gene localized on Xp22.1. In a knockout mouse model for XLRS, a proof of principle for treatment was given by demonstrating that the phenotype can be rescued using viral gene transfer of human RS1. These studies have mostly resorted to a rhodopsin proximal promoter for driving RS1 expression in retinal photoreceptors. To ensure an efficient but RS1-specific spatiotemporal expression in future efforts to treat XLRS in human, in depth knowledge of relevant *cis*-regulatory elements (CREs) is required. Therefore, we aimed to identify and validate functionally important CREs of the RS1 gene locus.

Methods: The phylogenetic conservation of putative CREs was determined and binding sites for the retinal transcription factor cone-rod homeobox (CRX) were predicted *in silico*. CRX sites were validated by chromatin immune precipitation (ChIP). *Cis*-regulatory activities of identified elements were assessed by luciferase assays and electroporation of dsRED reporters into explanted living mouse retinas.

Results: Sequence analysis and binding site prediction revealed an upstream CpG-island and several CRX consensus sequences within the proximal promoter and the first intron of the RS1 gene. ChIP assays confirmed that both regions were bound *in vivo* by CRX. Luciferase assays in cell cultures demonstrated that the minimal promoter region was essential for RS1 expression. Its activity was strongly enhanced by the CpG-island while the CRX bound region of the first intron exerted suppressive effects. dsRED reporters driven by the minimal RS1 promoter and the CpG-island were also highly active when electroporated into mouse retina confirming the *in vitro* findings.

Conclusions: Our *in vitro and ex vivo* data suggest that the minimal promoter region and the CpG-island are essential to drive high levels of RS1 gene expression. Furthermore, the CRX bound region of the first intron may mediate fine-tuning of gene expression. This suggests that future viral vectors designed for RS1 gene replacement therapy should include a minimal set of regulatory elements as defined in the present study.

Normoxic activation of HIFs in photoreceptors provides transient protection against light induced degeneration

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Purpose: Hypoxic preconditioning protects the retina against light induced photoreceptor degeneration. Transcription factors like hypoxia inducible factors (HIFs) are activated during hypoxia and regulate the expression of target genes that may play an important role in the protection of photoreceptors from damage. This study was performed to analyze whether normoxic activation of HIFs in photoreceptors could protect these cells similar to hypoxic preconditioning.

Methods: Von Hippel Lindau (VHL) protein was knocked down specifically in photoreceptors and after the development of the eye by breeding *Vhlh* $^{flox/flox}$ mice to mice expressing Cre-recombinase under the opsin promotor. 10-week-old *Vhlh* $^{flox/flox}$;opsin-cre mice were analyzed for HIF stabilization by Western blot and target gene expression by real-time PCR. Mice were exposed to bright white light and photoreceptor cell death was assessed 36 hours and 10 days post exposure.

Results: In 10-week-old *Vhlh* $^{flox/flox}$;opsin-cre HIF-1α and HIF-2α were stabilized and expression of target genes like adrenomedullin, STAT3 or metallothioneins was induced. However, expression of erythropoietin (EPO), that was thought to play a major role in hypoxia mediated neuroprotection, was not induced. Nevertheless light exposed *Vhlh* $^{flox/flox}$;opsin-cre mice showed a protection of photoreceptors at 36 hours after the insult. However, this protection was lost 10 days after light.

Conclusion: Normoxic activation of HIF transcription factors in photoreceptors transiently protects against light induced retinal degeneration. The lack of full protection as observed after hypoxic preconditioning indicates that hypoxia may induce protective factors in cells other than rod photoreceptors. These factors may include EPO and may act in a paracrine fashion to protect against degeneration. Alternatively, artificial activation of HIF transcription factors is not sufficient for a complete 'hypoxic' response in normoxia.

Keywords: hypoxic preconditioning, HIF, VHL, EPO, neuroprotection, light induced retinal degeneration.

Complement Regulation at Necrotic Cell Lesions is Impaired by the AMD Associated Factor H - H402 risk variant

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Purpose: Age related macular degeneration (AMD) is a common form of blindness in western societies. During the last years genetic analyses provided a strong link between AMD and the complement system, in particular for complement Factor H. A Tyrosine (Y) to Histidine (H) exchange at amino acid position 402 of the Factor H protein increases the risk for AMD significantly. However, the functional consequences of the Factor H and also the FHL1 sequence variation remain unclear. To this end we determined the relevance of the Y402H polymorphism under inflammatory conditions.

Results: Here, we describe that on the surface of necrotic retinal pigment epithelial cells distinct lesions appear, that are characterized as specific binding sites for monomeric CRP (mCRP). We show that at the surface of necrotic cells mCRP is generated from cell bound pentameric CRP. mCRP recruits the complement regulators Factor H and FHL1 to these distinct lesions as shown by flow cytometry and immunofluorescence microscopy. The bound regulators accelerate complement inactivation and enhance iC3b deposition on the necrotic cell surface. At the same time the Factor H - mCRP complex restricts the inflammatory response, such as the release of the pro-inflammatory cytokine TNF-a by macrophages. This novel mechanism of enhanced mCRP-mediated complement inhibitory and anti-inflammatory activities of Factor H and FHL1 at necrotic lesions is affected by the AMD associated sequence variation at amino acid position 402. The H402 risk variants show reduced binding to mCRP and this correlates with significantly reduced surface recruitment to necrotic lesions, reduced local complement regulation and enhanced pro-inflammatory processes.

Conclusion: Diminished local complement control at retinal surfaces such as the retinal pigment epithelium and the Bruchs membrane, can promote accumulation of necrotic debris, which results in a chronic local inflammation, Drusen formation and AMD progression.

A cell culture based screening system for the identification of cone neuroprotective compounds

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Purpose: Human vision critically depends on cone photoreceptor function. Cone degeneration is the hallmark of many retinal diseases such as age-related-macula-degeneration (AMD), diabetic retinopathy, retinitis pigmentosa, achromatopsia, and cone-rod-dystrophies and, to date, no or only inadequate treatment is available. The search for neuroprotective compounds to prevent or delay cone cell death requires a reliable test system that is convenient to use and allows for high-throughput screening of many different drugs at once. Here, we aim at establishing a cell culture based screening system using the cone-like 661W cell line.

Method: We used immunofluorescence methods to characterize cultured 661W cells. These cultures were subjected to different stress paradigms designed to induce cell death in ways that mimic specific inherited forms of photoreceptor degeneration. To this end different PDE inhibitors, PKG activators, and pro-oxidants were used and the outcome assessed using viability assays such as TUNEL and alamarBlue. In addition, specific markers for degenerative events (e.g. cGMP accumulation, lipid peroxidation) were analyzed using immunhistochemical techniques.

Results: 661W cells were found to express a number of cone photoreceptor specific markers including cone opsins, glycogen phosphorylase and the cone specific PDE6. Importantly, rod photoreceptor markers were not expressed. PDE inhibitor treatment resulted in increased intracellular cGMP levels and cell death. Similarly, PKG activation and oxidative stress decreased cell viability.

Conclusions: The cone-like status of 661W cells and the expression of cone specific marker proteins was confirmed. These cells reacted to different stress paradigms in ways similar to photoreceptors *in vivo*, suggesting the presence and activity of similar cell death mechanisms. Taken together, 661W cell based test systems may be useful to mimic crucial aspects of cone photoreceptor neurodegeneration and neuroprotection.

Interaction of L-type Ca^{2+} channels and Ca^{2+}-activated K^+ channels plays a role in the phagocytic function of the retinal pigment epithelium

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Objective: In the retinal pigment epithelium (RPE) the activation of L-type Ca^{2+} channels regulates different physiologically important cell functions like phagocytosis. Recently a direct coupling of large-conductance voltage- and Ca^{2+}-activated K^+ channel (BK channel) activity to L-type Ca^{2+} channel activation and a negative feedback control of these Ca^{2+} channels have been described (Wimmers et al. 2008, Molecular Vision). Aim of this study was to investigate the relevance of these interactions for phagocytosis.

Methods: To analyze the role of BK channels in phagocytosis mice deficient for the pore-forming channel protein of BK channels were used (BK-/-). For in vivo measurement of circadian rhythm of retinal phagocytosis we labeled the outer segment protein rhodopsin in retinal cross-sections of mice and quantified rhodopsin-positiv phagosoms in the RPE at two time points during the day: 30 minutes and 8 hours after onset of light in the morning.

Results: In the morning (30 min after onset of light) phagocytic activity showed in the wildtype mice a peak of 11±4 phagosomes/ 100µm retina which decreased to a base level of 4±2 phagosomes/ 100 µm (8h after onset of light). In the BK-/- mice we observed an increase of the peak activity to 15±5 phagosomes / 100µm and a return to lower base level of 2.5±2 phagosomes/ 100µm. Both, the peak phagocytic activity in the morning and during the afternoon were significantly different ($P < 0.001$). Additionally we observed in the BK-/- mice a decrease in photoreceptor outer segment length, where we measured a ratio outer segment length/ inner segment length of 1.83±0.6 compared with 2.23±0.8 in wildtype mice. Conclusion: In conclusion, the absence of BK channels lead to altered circadian regulation of phagocytic function of the RPE. These data indicate for the first time a role of ion channels in the regulation of phagocytosis by the RPE.

Different pericentrin-isoforms identified in mammalian photoreceptor cells

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Pericentrin (PCNT), a conserved protein of the pericentriolar material (PCM), plays an important role in microtubule organization and serves as a multifunctional scaffold for numerous proteins and protein complexes. PCNT interacts with various proteins and in this way contributes to a diversity of fundamental cellular processes. Recent studies indicate that PCNT mutations are associated with a range of diseases, e.g. Majewski/microcephalic osteodysplastic primordial dwarfism type II (MOPDII) and Seckel syndrome, two rare human autosomal recessive genetic disorders. Moreover, further studies suggest a role for PCNT in human cancer, mental disorders and ciliopathies.

Until today two PCNT splice variants from orthologous genes in mice and humans – PCNT 250 and 360 (human 380) – are known. The proteins are characterized by coiled-coil domains throughout most of their structure and both contain a PCM targeting motif called the PACT domain. PCNT localizes also to the base of primary and motile cilia and is involved in cilia development and cilary function in mammalian cells.

In the vertebrate retina, photoreceptor cells are morphologically and functionally arranged in several compartments. The light sensitive photoreceptor outer segment is linked with an inner segment, which contains the typical energy producing and protein synthesizing components of an eukaryotic cell, via a modified, non-motile cilium, termed the connecting cilium. Using various methods, e.g. laser microdissection in combination with RT-PCR, immunocytochemistry, western blotting and real-time PCR we find that all known PCNT-isoforms are expressed in photoreceptor cells. PCNT and several centrosomal interaction partners are localized at the basal body and the centriole of the connecting cilium. Here, PCNT also co-localizes with the whole machinery which is involved in protein transport from the inner to the outer segment. Moreover, we find in our western blotting experiments with different mouse tissues, that the PCNT isoforms are expressed in varying intensitys between the different tissues and between the isoforms. This fact suggests distinct functional roles of the different isoforms – also in photoreceptor cells.

The presence of PCNT at the connecting cilium, the site of transport regulation and interaction with transport molecules like IFTs, suggests a role of PCNT in ciliary transport in photoreceptor cells. Studying PCNT function, especially of the different isoforms, will provide novel insights into human disorders related to defects in ciliary function.

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HDAC activity is causally involved in *rd1* mouse photoreceptor cell death

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Purpose: Retinitis Pigmentosa (RP) is a blinding disease in which photoreceptors are lost due to specific gene mutations. RP is at present untreatable and the underlying neurodegenerative mechanisms are largely unknown. Post-translational modifications of histones are known to affect cell death and survival and such modifications may be introduced by histone acetyl transferases (HATs) and histone deacetylases (HDACs), which mediate acetylation and deacetylation, respectively. The aim of this study was to investigate the interplay between histone acetylation and deacetylation and to study their relevance for photoreceptor survival in the *rd1* mouse model for RP.

Methods: Retinae were obtained from *rd1* and *wt* animals either directly or after *in vitro* culture and treatment with inhibitors specific for HDACs. Protein acetylation and poly-ADP-ribosylation was studied using immunofluorescence and western blot. HDAC expression was studied using immunofluorescence and micro-array techniques. Cell death was assessed using TUNEL assay. Custom developed *in situ* activity assays for HDACs and PARP were performed to study activity of different classes of these enzymes in *rd1* and *wt* retinae.

Results: We found acetylation of histones and other proteins to be dramatically reduced in degenerating photoreceptors in the *rd1* mouse retina. At the same time poly-ADP-ribosylation was strongly increased. The activity assays for HDAC and PARP showed a positive reaction in *rd1* photoreceptors, when compared with *wt*. Activation of HDAC class I/II temporally preceded PARP activity and photoreceptor degeneration. Inhibition of HDAC class III (sirtuins) did not have a positive effect on retinal degeneration while inhibition of HDAC class I/II reduced activation of PARP and increased *rd1* photoreceptor survival *in vitro.*

Conclusions: HDACs are likely to be involved in the development and progression of photoreceptor cell death in the *rd1* mouse and may directly or indirectly trigger activation of PARP. HDAC class I/II may be more important for degenerative processes than sirtuins. These findings highlight the importance of protein acetylation for photoreceptor cell death and survival and propose certain HDAC classes as novel targets for the pharmacological intervention in RP.

Enrichment of photoreceptor precursors via Fluorescence Activated Cell Sorting (FACS) and transplantation into the subretinal space of adult mice

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Introduction: A possible option for treatment of retinal degeneration diseases like Retinitis pigmentosa (RP) and age-related macular degeneration (AMD) might be a cell replacement strategy. The aim of this work is to replace degenerated photoreceptors by transplanting retinal precursors that differentiate into functional photoreceptors. To increase the integration rate of transplanted cells into the outer nuclear layer (ONL) we enriched retinal precursors via FAC-sorting for specific rod precursor markers. By transplanting enriched rod precursor populations with decreased contamination of other cells instead of a mixture of all retinal cells the integration rate might increase.

Methods: We isolated retinas of early postnatal NrlGFP$^{+/-}$:aDsRed$^{+/-}$ and rhoGFP$^{+/-}$:aDsRed$^{+/-}$ mice (postnatal day 2 - 6), Trypsin- (0,05 % in PBS) digested (20 min, 37°C) and mechanically dissociated them by gentle pipetting. Cells (20x10^{6}cells/ml) were sorted with BD FACSAria II^{TM} for EGFP positive and negative fractions. They were either seeded in culture medium (DMEM/F12, 1% NCS) on laminin coated PLL-coverslips or transplanted (1 µl with 200.000 cells/µl) into the subretinal space of adult C57BL/6 wt mice. After 4 days seeded cells were fixed with 4% PFA and DAPI-stained. Mice with transplanted cells were euthanized after 3 weeks and perfused with 4% PFA. Retinas were removed, agarose embedded and sectioned with a vibratome.

Results: After FAC-sorting we obtained a successful enrichment of photoreceptor precursors with a purity of 85 to 95%. Long sorting time and mechanical stress leads to appearance of dead cells during reanalysis. Cultivation shows survival and enrichment of GFP positive cells. Despite FAC-sorting lots of cells remain after transplantation into wt hosts in the subretinal space and only a few integrates into the ONL. Integrated cells survived and formed photoreceptor like morphology.

Conclusion: Enrichment of photoreceptor precursors via FACS is possible and after transplantation into retina cells integrate into the ONL and form morphological mature photoreceptors. However, a lot of cells don't survive sorting procedure and still only a minority of cells do actually integrate. These cells could be a subpopulation of photoreceptor precursors which might be sorted with more specific markers. Also disruption of the outer limiting membrane (OLM) which might represent a barrier for integration could increase the rate of integrated cells in future experiments.

Phenotypic characterization of a bestrophin-1 knock-out mouse

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Background: Bestrophin-1 (BEST1) encodes an integral membrane protein of the retinal pigment epithelium (RPE). Mutations in this gene are associated with Best vitelliforme macular dystrophy (BVMD), an autosomal dominant form of macular degeneration with highly variable expressivity and reduced penetrance. A key feature of the disease is a striking accumulation of lipofuscin-like deposits in the RPE, degeneration of photoreceptors, impairment of visual function and a reduced light-peak in the electrooculogram. To gain insight into the function of BEST1 and its role in the pathophysiology of BVMD, we have generated a knockout mouse line deficient in BEST1. Here we present a detailed phenotypic characterization of the BEST1$^{-/-}$ mouse.

Methods: BEST1$^{-/-}$ mice were generated by disruption of the BEST1 gene in exon 6. Northern Blot analysis was used to test transcription of the targeted locus and deficiency of the BEST1 protein was confirmed by Western Blot analysis. Differently regulated gene expression was investigated by DNA microarray analysis (Affymetrix Exon Array). Histological examination of retina sections was done by light and electron microscopy. Visual acuity was determined by using a virtual optomotor system (*OptoMotry*, *CerebralMechanics*). To investigate the retinal lipid metabolism, lipid composition was analysed by GC-mass-spectrometry. RPE-phagocytosis was determined by measuring the diurnal shedding and uptake of photoreceptor outer segments. Towards this goal, RPE was flat mounted, stained with anti-Zonula-occludens-1 and anti-Rhodopsin-1D4 and 1D4-positive phagosomes were counted. In addition, expression of genes involved in RPE-phagocytosis was tested by quantitative RT-PCR.

Results: Compared to wild type littermates, BEST1$^{-/-}$ mice show complete BEST1 deficiency. Microarray analysis reveals no differently regulated genes and neither histological analysis nor the measured visual acuity shows significant differences between the genotypes. Both genotypes do not differ in their retinal lipid composition and no sign of an impaired RPE-phagocytosis can be detected, as shown by quantitative uptake analysis of photoreceptor outer segments and expression of genes involved in RPE-phagocytosis.

Conclusions: Despite BEST1 deficiency, the molecular and histological phenotype of BEST1$^{-/-}$ mice reveals no sign of impaired retinal function. The absence of bestrophin-1 appears well toler-

ated in this tissue. This raises the possibility of functional redundancy by other members of the bestrophin family. Understanding bestrophin-1 function and its pathophysiological role in disease will be a key for developing a therapeutic treatment in BVMD.

Characterization of the retinal phenotype in PARP-1 KO animals

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Purpose: Poly (ADP-ribose) polymerase (PARP)-1 is an abundant nuclear enzyme that is activated by DNA strand breaks. It is a member of a large family of enzymes that use NAD+ as a substrate to transfer ADP-ribose onto an acceptor protein. PARP-1 deficient mice and cells are very sensitive to both ionizing radiations and alkylating agents, thus supporting a role for PARP-1 in the cellular response to DNA damage. PARP over activation was found to be involved in photoreceptor degeneration in the *rd1* mouse model for Retinitis Pigmentosa. The aim of this study was to characterize the phenotype of PARP-1 KO retinae.

Methods: PARP-1 knockout (KO), *rd1* and corresponding wild-type (wt) animals at post-natal day (P) 11 and P30 were used. Each group included at least 3 different animals. TUNEL staining to detect dying cells was performed on cryosectioned retinae. Hematoxylin-Eosin staining, cGMP staining, PARP, Calpain, HDAC activity assays and PAR immunohistochemistry were performed for analysis of retinal structure, cGMP level, PARP, Calpain, HDAC activation, PAR accumulation, respectively.

Results: Hematoxylin-Eosin staining showed a similar structure and retinal thickness for PARP-1 KO and wt at P30. Contrary to *rd1,* PARP-1 KO and *wt* retinae showed very few cells positive for an accumulation of cGMP in the outer nuclear layer (ONL). TUNEL assay for detection of degenerating cells showed less positive cells in the ONL of both PARP-1 KO and *wt* retinae when compared to *rd1*. Similar results were obtained when specific enzymatic assays were used for detection of PARP, calpain, HDAC activation, PAR accumulation respectively.

Conclusion: PARP-1 KO retinae appeared morphologically normal and did not exhibit any enzymatic activities associated with retinal degeneration. These results suggest that neuroprotective strategies aimed at inhibiting PARP activity would not *per se* negatively affect retinal cell survival.

Investigations on the allelic frequency of certain recurrent mutation in *CNGB3* and *ABCA4*

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Purpose: Achromatopsia is a congenital, autosomal recessively inherited disorder characterized by lack of color discrimination, low visual acuity, photophobia and nystagmus. Mutations in four genes *CNGA3* (20-30%), *CNGB3* (40-50%), *GNAT2* (<2%) and *PDE6C* (>2%) have been associated with this disorder, and a 1bp-deletion c.1148delC in *CNGB3* is the most prevalent mutation, accounting for ~75% of all *CNGB3* mutant alleles.
Mutations in *ABCA4* are the cause of autosomal recessive Stargardt disease, but also autosomal recessive cone-rod dystrophy and autosomal recessive retinitis pigmentosa and several mutations are observed recurrently in different populations. In a study conducted in Tübingen, the most frequent mutation was c.5461-10T>C, a mutation with unclear pathogenicity, while in other studies the most common mutation was c.2588G>C that was found with an overall frequency of up to 1:54 in the general population.
At the moment almost no data on the prevalence of genes and frequency of certain common mutations for inherited retinal dystrophies are available. Yet this information is necessary for adequate genetic counseling and estimation of recurrence risk.

Methods: We analyzed a control panel of 500 healthy probands via PCR/RFLP (restriction fragment length polymorphism) for the mutation c.1148delC in *CNGB3* and the mutations c.1622T>C, c.2588G>C via high resolution melting (HRM) and c.5461-10T>C via PCR/RFLP in *ABCA4* and followed by sequencing to confirm found sequence variants.

Results: In our panel of 500 control probands, we identified two subjects that were heterozygous for the mutation c.1148delC in the *CNGB3* gene, implicating a prevalence of this common mutation of 1:250 in the general population. For *ABCA4*, no proband carried the mutation c.1622T>C, nor c.5461-10T>C, while the mutation c.2588G>C was seen heterozygously in three probands, estimating a prevalence of this mutation of 1:166.

Conclusion: The data for the mutation c.1148delC in *CNGB3* match with a calculated frequency of 1:250 according to the Hardy Weinberg Equilibrium. The data for *ABCA4* show that allele frequencies vary substantially between different populations, and confirm that c.2588G>C is commonly found in the general population. And the exclusion of c.5461-10T>C in 500 controls again supports an impact of this unclear mutation for ABCA4-related retinal disease.

Combined mRNA and miRNA expression profiling of the *CNGA3*$^{-/-}$ mouse – a mouse model of achromatopsia

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Purpose: The *CNGA3*$^{-/-}$ mouse is an animal model lacking the A subunit of the cone specific cyclic nucleotide gated channel. The phenotype is characterized by a loss of cone photoreceptor function and a progressive degeneration of the cones. To elucidate the biological events leading to the loss of photoreceptors we combined mRNA expression experiments with whole genome miRNA expression profiling.

Methods: Expression analysis of *CNGA3*$^{-/-}$ and wildtype retinas in 2 age stages was performed using Affymetrix MOE 430 2.0 microarrays. Differential regulated transcripts with a minimum change in expression level of 1.5 fold (p-value ≤0.05) were obtained and gene regulation networks were generated by the Ingenuity Pathways Analysis software. To verify the data 10 transcripts per time point were analyzed by qRT-PCR. miRNA expression profiling was conducted on an Illumina whole genome mouse miRNA array.

Results: 496 transcripts were differentially regulated in the retinas of the 4 week old mice and 204 in those of the 8 week old animals. Gene regulation networks revealed misregulations of genes associated with RNA post-transcriptional modification and cellular growth. 80 % of the transcripts chosen for real-time validation could be verified. In the miRNA array analysis of 4 week old mice we found 97 differently regulated miRNAs which have potential target genes included in the differential gene list of our previous transcriptional analysis. Three miRNAs were validated by qRT PCR including miRNAs linked to RNA post-transcriptional modification or ophthalmological diseases.

Conclusions: Expression analysis of the *CNGA3*$^{-/-}$ mouse highlighted a misregulation of the phototransduction cascade in accordance with the loss of visual function that characterizes the phenotype. The combination of mRNA and miRNA expression profiling permits a closer monitoring of the neurodegenerative events in the retina occurring during the course of degeneration.

In vitro fostering of postmitotic photoreceptors

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Cell replacement strategies such as stem cell transplantation are considered as a therapeutic approach for retinal photoreceptor degeneration, one of the major causes for vision impairment world wide. Understanding the mechanisms of survival, integration and maturation of photoreceptors are crucial aspects for regenerative therapies besides stem cell expansion and commitment to photoreceptor fate. However, many questions can be poorly addressed *in vivo*. Here we present *in vitro* approaches and the advantages of biomaterial scaffolds to investigating survival, maturation and integration potential of postmitotic photoreceptors.
Survival and maturation of a mixed retinal cell population and enriched postmitotic photoreceptors (~80% purity) isolated from rhodopsin-green fluorescent protein (rhoGFP) transgenic mice at postnatal day 4 were tested on various surface coatings and under different culture conditions. Furthermore, the growth of photoreceptors on bioartificial scaffolds mimicking the nanoscale spatial organization of the extracellular matrix collagen TypeI has been investigated.
Retinal cells showed increased survival and settling properties when cultured in presence of 1%NCS or FGF2. The survival of enriched photoreceptors highly depends on a dense cell seeding (^{3} 1 Mio cells/cm^2), which suggest the requirement of cell/cell contacts. Survival and attachment of the photoreceptor fraction can be improved when coating hydrophobic surfaces such as poly-d-lysine (PDL) and poly(octadecene-alt-maleic acid) (POMA) with FGF2 but not NCS. On aligned collagen fibrils photoreceptors showed growth of oriented protrusions with an increased number and length in presence of FGF2. Collagen fibrils might be crucial for guidance of photoreceptor protrusion as shown for neurites in neural development and repair.
In conclusion the isolation of photoreceptors from a mixed population is important to further study their properties. Enriched photoreceptors are very sensitive and require well defined *in vitro* culture conditions. Biomaterials will help to support their survival and study their maturation and integration potential (e.g. orientation and migration) *in vitro* in combination with extrinsic factors or hosting cells. Biomaterials can be used for the development of *in vitro* assays to further address these questions.

The USH1G protein SANS interacts with the dynein-dynactin motor component p150^{Glued} and the ciliopathy related CEP290

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Purpose: The human Usher syndrome (USH) is the most common form of combined deaf-blindness and clinically divided into 3 subtypes (USH1-3). Mutations in USH proteins lead to profound inner ear defects and retinal degeneration. For the function and maintenance of photoreceptor cells vectorial transport processes are essential. We previously identified protein networks related to the human USH as components of vesicle transport between the inner and outer segment of photoreceptor cells. The USH1G protein SANS (scaffold protein containing ankyrin repeats and SAM domain) is a main organizer of this ciliary-periciliary network. Here, we adressed the function of SANS in the USH protein interactome and in transport processes in photoreceptor cells.

Methods: Identification of SANS interactors in yeast-2-hybrid screen (Y2H). Validation of interaction by GST-Pulldown and fluorescence resonance energy transfer (FRET). Localization studies via light microscopy.

Results: SANS is located at microtubule rich regions of photoreceptor cells: the apical inner segment, the connecting cilium and its associated centriole. SANS' localization depends on an intact microtubule network in NIH3T3 and murine photoreceptor cells. Y2H sreens with SANS central domain (CENT) identified 30, mainly ciliary or transport associated proteins. The direct interaction between the ciliopathy related CEP290 (centrosomal protein 290 kDa) and the dynein-dynactin motor component p150^{Glued} with SANS was verified. These interactions are confirmed by co-localization studies in HEK293T cells and cryosections of murine retina.

Conclusions: Present results identified p150^{Glued} and CEP290 as novel components of the USH protein network via direct interaction with SANS. p150^{Glued} mediates cargo binding to the motor protein dynein. According to our hypothesis, this allows an involvement of SANS in intracellular transport processes. The direct interaction between SANS and CEP290 molecularly bridges the USH to a broad spectrum of ciliary deseases. Defects in both proteins lead to a variety of ciliary defects like *retinitis pigmentosa*. Taken together, the ciliary proteins CEP290 and p150^{Glued} provide the direct molecular linkage between USH to other ciliopathies and to intracellular transport mechanisms via SANS.

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Establishment of a calcium imaging based bioassay for the identification of chemical compounds that improve surface expression of trafficking deficient mutant CNGA3-channels

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Purpose: Achromatopsia, an autosomal recessive inherited retinal disorder, is characterized by a strongly reduced visual acuity, lack of color discrimination, photophobia and a nystagmus. The majority of patients carry mutations in *CNGA3* and *CNGB3*, which encode for the A3 and B3 subunit of the cone cyclic nucleotide gated (CNG) channel. CNG channels are a crucial component of the phototransduction cascade and enable the cGMP-dependent influx of sodium and calcium into the cone photoreceptor outer segment. Several *CNGA3* mutations have been found to interfere with protein trafficking and/or folding and thus result in a decreased channel density in the plasma membrane in heterologous expression systems. We established a bioassay, allowing a high-throughput screening for chemical and pharmacological chaperones, which may help to overcome the trafficking/folding deficits of mutant CNG channels.

Methods: Plasmids encoding for the wild type as well as the mutant CNGA3 channels R427C and R563C were co-transfected with the pCAeq plasmid (encoding for the apoaequorin) in HEK293 cells. Aequorin is a calcium sensitive complex, which upon calcium binding oxidizes its bound coelenterazine to coelenteramide, resulting in the emission of light and the release of the coelenteramide. Transfected cells were treated with chaperones for 24 hours and subsequently the emitted light prior and after activation of the CNG channels with 8-Br-cGMP were recorded in a luminometer.

Results: Measurements of HEK 293 cells expressing wild type or mutant channels revealed that the maximal luminescence signal of the two mutants R427C and R563C was significantly lower compared to the wild type channel. An improvement in the maximal luminescence signal of both mutated channels was observed after the treatment of transfected cells with glycerol. Three additional chemical chaperones (6-aminohexanoic acid, TMAO and Sorbitol), that have been tested so far, had no effect onto protein trafficking/folding.

Conclusion: The assay is suitable for the screening of substances, which could improve the trafficking/protein folding of mutant CNG channels. Treatment of cells with glycerol, a known chemical chaperone, improved the luminescence signal of the two analyzed mutants, indicating that the assay is sensitive and effective for a compound screening strategy.

Differential ciliary and non-ciliary localization of IFT molecules in the mammalian retina

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Purpose: The assembly and maintenance of cilia require intraflagellar transport (IFT), a process mediated by molecular motors and IFT particles composed of individual IFT proteins. Although IFT is related to several ciliopathies, including eye diseases, and is in focus of current intense research the spatial distribution of individual IFT proteins remained so far elusive. Here, we analyzed the expression and subcellular localization of individual IFT proteins in retinal cells. Knowledge of the precise spatial distribution of IFT proteins in the retinal photoreceptor cells should provide valuable novel insights into cellular functions of retinal IFT and their role in photoreceptor cell biology in health and disease.

Methods: We studied the expression and the subcellular localization of individual IFTs, namely IFT20, 52, 57, 88, and 140 in the mouse retina applying Western blots and recently introduced correlative high resolution immunofluorescence and immunoelectron microscopy.

Results: We showed that IFT proteins are expressed in the retina. In photoreceptor cells IFT proteins were differentially localized in sub-compartments of photoreceptor cilia and in periciliary target compartments of the cytoplasmic transport through the inner segment. In the inner segment IFT molecules were regularly associated with transport vesicles. Interestingly, IFT20 was not in the IFT core complex in photoreceptor cilia but was accompanied Golgi-based sorting and vesicle trafficking of ciliary cargo. Moreover, we identified a non-ciliary IFT system containing a subset of IFT proteins in dendrites of retinal neurons, namely bipolar and horizontal cells.

Conclusions: In our present study we shine new light on IFT in the retina. Our data indicate the presence of IFT complexes different from the ones previously described in lower eukaryotes. Two different sets of IFT proteins were found to be associated with distinct transport routs of ciliary cargos to the outer segment. Furthermore, we grounded evidence for a role of IFT proteins in vesicular transport in dendrites of non-ciliated retinal neurons. Collectively, our data implicate the differential composition of IFT systems in cells with and without primary cilia, thereby supporting new perspectives on IFT function beyond its well established roles in cilia. Moreover, we provide novel insights into the protein networks underlying ciliopathies.

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GFAP and Vimentin Deficient Mice show altered expression of inwardly rectifying potassium channel Kir4.1 and retinal function

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Purpose: Müller cells and astrocytes show strong upregulation of the intermediate filaments glial fibrilary acidic protein (GFAP) and vimentin following stress in the retina. The lack of these filaments has been shown to alter the reactivity of glial cells to numerous insults. The purpose of this study was to examine the retinal function of GFAP/vimentin-KO mice under normal physiological conditions.

Methods: Retinal function of GFAP/vimentin-KO mice, of single KO (GFAP or vimentin), and of congenic controls was analyzed by electroretinography (ERG). The expression and distribution of neuronal and glial markers, potassium-channels, and water-channels were examined with immunohistochemistry and western blot.

Results: When comparing GFAP/vimentin-KO mice and control or single KO mice, no difference was observed in the a-waves of their ERGs. Scotopic b-wave amplitudes, on the other hand, were significantly higher in GFAP/vimentin-KO mice in all ages analyzed. The distribution and levels of most examined neuronal and glial markers were similar in all groups. In contrast, the expression of the inwardly rectifying potassium channel (Kir) 4.1 was markedly reduced in the proximal processes of the Müller cells of GFAP/vimentin-KO mice.

Conclusion: The inability to express GFAP and vimentin affects the distribution of Kir4.1 channels on Müller cells. This could be due to a defective transport of the protein in these cells or to an alteration in the targeting to or the stabilization of Kir4.1 in the inner portions of the cells. A reduction of Kir4.1 is likely to affect the potassium buffering capacity of these cells, which may in part account for the ERG findings.

Effect of TIMP3 mutation S156C on proteasome activity

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Purpose: Mutations in the gene encoding tissue inhibitor of metalloproteinase-3 (TIMP3) cause Sorsby fundus dystrophy (SFD), a late-onset macular degeneration initiated by abnormal thickening of Bruch's membrane. Mutant TIMP3 molecules tend to dimerize and may have an increased susceptibility to oxidative damage. Misfolded or oxidized proteins are degraded by the cellular ubiquitin-proteasome system. The objective of this study was to determine the impact of the S156C-TIMP3 mutation on proteasome activity.

Methods: Primary lung fibroblast and retinal pigment epithelial (RPE) cells were derived from wild-type, *Timp*$^{S156C/+}$ and *Timp*$^{S156C/S156C}$ mice. Fibroblasts were exposed to cellular stress by incubation without serum and addition of hydrogen peroxide. Cell viability was determined by MTT assay. Peptidase activities of the proteasome were measured using fluorogenic peptides.

Results: Chymotrypsin-like, trypsin-like and peptidylglutamylpeptide hydrolase enzyme activities were all observed to be significantly higher in *Timp*$^{S156C/+}$ and *Timp*$^{S156C/S156C}$ derived cells when compared to wild-type cells after serum deprivation.

Conclusions: Our results indicate that the endogenous expression of S156C-TIMP3 mutant proteins leads to an elevation of proteasome activity which may be attributed to an accumulation of misfolded proteins in the mutant cells.

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