PRO RETINA FOUNDATION
FOR PREVENTION BLINDNESS

4th PRO RETINA
Research-Colloquium Potsdam

CONFERENCE REPORT

Retinal Degeneration

Cones in Focus

An Interdisciplinary Dialogue

March 28th/ 29th, 2008

Potsdam, Seehotel am Templiner See

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 65 regional groups, which are spread throughout Germany.
At present (2008), PRO RETINA Deutschland e.V. counts more than 6,200 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 March 28th 2008:

13:00-13:05 Franz Badura (ProRetina Research Foundation, research division)
Welcome and opening remarks

13:05-13:15 Christina Fasser, Zürich (President of Retina-International):
"Patients and scientists – An international partnership"

13:15-13:30 Eberhart Zrenner, Tübingen (Chairman of the German Scientific Medical Advisory Board):
"Retinal Degeneration – Cones in Focus"

13:30-15:10 **Session 1, chairman Prof. Dr. Klaus Rüther: Cone/Cone-Rod dystrophies: Clinical and Genetic Aspects**

1. Ulrich Kellner, Siegburg: "Clinics of cone dominated retinal dystrophies"
2. Hanno Bolz, Cologne: "Genetics of cone/cone-rod dystrophies and deafness"
3. Veronique Kitiratschky, Tübingen: "Genetics of cone and cone/rod dystrophies"
4. Sandra Brunner, Zürich: "Cone versus rod disease in RPGR mutant mouse lines"

15:10-15:50 Coffee break and scientific chitchat

15:50-17:30 **Session 2, chairman Prof. Dr. Wolfgang Baehr: Pathophysiology in Cone/Cone-Rod Dystrophies**

1. Wolfgang Baehr, Salt Lake City: "Mechanisms of cone degeneration"
2. Oliver Biehlmaier, Zürich: "The cone dominated zebrafish retina as a model for retinal disease"
3. Michel Michaelides, London: "Cone/cone-rod/macular dystrophy phenotypes associated with GCAP1 mutations"
4. Irina Golovleva, Umea: "Non-receptor tyrosine kinase mutations and cone dystrophy"

18:30 Dinner

19:30 "Swingin' Poster Session and Get Together"

PROGRAMME

Saturday March 29th 2008:

8:30-10:10 **Session 3, chairman Prof. Dr. Olaf Strauss: Hereditary Macular Degeneration**

1. Thomas Loenhardt, Regensburg: "Retinoschisin deficiency in macular degeneration"
2. Vladimir Milenkovic, Regensburg: "Best macular dystrophy: Mechanisms of Ca^{2+} regulation"
3. Jan Wijnholds, Amsterdam: "CRB1 viral gene therapy to prevent onset of Leber congenital amaurosis"
4. Simone Kellner, Siegburg: "Fundus autofluorescence and near-infrared autofluorescence in cone and macular dystrophies"

10:10-10:45 Coffee break

10:45-12:25 **Session 4, chairman Prof. Dr. Bernhard Weber: Genes and Mechanisms in AMD pathogenesis**

1. Lars Fritsche, Regensburg: "New insight into LOC387715-associated AMD"
2. Peter Zipfel, Jena: "The Ying and Yang of the Complement"
3. Hendrik Scholl, Bonn: "Systemic complement activation in AMD"
4. Caroline Klaver, Amsterdam: "ApoE metabolism and AMD"

12:25-13:00 **Poster Awards and Short Presentations**

13:00 Lunch and end of meeting

SPEAKER ABSTRACTS

Clinics of cone dominated retinal dystrophies

Ulrich Kellner

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Cone dominated retinal dystrophies are a heterogeneous group of retinal disorders which have early visual acuity loss, reading difficulties, color vision deficiency, central scotomata and photophobia in common. In clinical practice, these inherited disorders have to be distinguished from the much more frequent inflammatory, degenerative, vascular or toxic macular degenerations. In addition, for decades the clinician has tried to separate the inherited disorders into macular dystrophies (involving only the macula throughout the course of the disease), cone dystrophies (which predominately affect cones throughout the course of the disease) and cone-rod dystrophies (which involve cones and, later on, also rods and can not be distinguished from retinitis pigmentosa in the late stage of the disease).
Important basic diagnostic techniques are taking of the patients and family history, visual acuity, visual field testing and ophthalmoscopy. Ophthalmoscopy can be normal in early disease stages of macular dystrophies and even in more progressed stages of cone dystrophies. Therefore further diagnostic test are important to support the diagnosis. The full-field ERG allows to distinguish between macular dystrophies with normal ERG and cone- or cone-rod dystrophies with reduced cone-responses. In the last decade the multifocal ERG, fundus autofluorescence and optic coherence tomography (OCT) have proven useful techniques especially for the early diagnosis of macular and cone dystrophies.
Today the detailed clinical work-up is combined with molecular genetic analysis to facilitate the differential diagnosis between the several cone dominated retinal dystrophies and to establish the diagnosis for the individual patient. However, the combination of clinical and molecular genetic data does not allow us to answer the patient's most important question: to predict the future course of the disease. The huge variability of disease expression requires an extensive diagnostic approach and individual counseling for each patient.

Genetics of cone/cone-rod dystrophies and deafness

Hanno Jörn Bolz

Institute of Human Genetics, University of Cologne, Cologne, Germany

Purpose: To review the genetic conditions that can present with both, cone-rod degeneration and hearing impairment.

Methods: An overview is given based on the current literature as well as on our own studies.

Results: Cone-rod degeneration and hearing impairment is frequently observed in Alström syndrome and Refsum Disease. In Usher syndrome, retinitis pigmentosa occasionally progresses to cone dysfunction. Similarly, X-linked RP due to *RPGR* mutations may affect central vision and manifest with additional hearing loss. Moreover, cone disease and deafness exist in rare conditions such as mitochondrial disease, thiamine-responsive megaloblastic anemia syndrome (TRMA) and cutis verticis gyrata, retinitis pigmentosa and sensorineural deafness syndrome (MIM #605685). Importantly, the overlap of two non-syndromic genetic entities can mimic a cone-rod dystrophy-deafness syndrome as will be demonstrated. This should be taken into account, in particular if the parents of patients are consanguineous. Strategies that can be applied to uncover the respective genetic basis will be illustrated.

Conclusion: A combination of cone-rod dystrophy and deafness can be found in different genetic entities. The elucidation of the genetic cause is important for genetic counseling as well as for early therapeutic intervention and supporting strategies.

Genetics of cone and cone/rod dystrophies

Veronique Kitiratschky, Tübingen

Inherited progressive cone rod dystrophies (CRD) are characterized by progressive loss of cone photoreceptor function followed by progressive loss of rod photoreceptor function, often accompanied by retinal degeneration. In contrast, in inherited progressive cone dystrophies (CD) only cone function is impaired and retinal degeneration is often minimal and confined to the macula. All modes of Mendelian inheritance have been observed and several genes identified. However, numbers regarding the prevalence of gene mutations causing autosomal dominant or autosomal recessive CD and CRD vary considerably between studies or have been collected in relatively small cohorts. Especially for autosomal dominant CD and CRD there has been a lack of robust information regarding the frequency of disease causing gene mutations.
The aim of this study was therefore to determine the prevalence of gene mutations for autosomal recessive CD and CRD and autosomal dominant CD and CRD in a group of 64 and 27 independent patients, respectively.
We present selected data on the mutation analysis of the *ABCA4* and *GUCY2D* genes.

Cone versus rod disease in a mouse model for X-linked retinitis pigmentosa.

Sandra Brunner[1], Sergej Skosyrski[2], John Neidhardt[1], Renate Kirschner-Schwabe[3], Klaus-Peter Knobeloch[4], Elvira Rohde[4], Ivan Horak[4], Klaus Rüther[2] and Wolfgang Berger[1]

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Purpose: To understand the pathophysiology and the molecular mechanisms leading to retinal degeneration in two mouse strains (C57BL/6 and BALB/c) carrying an in-frame deletion of exon 4 of the retinitis pigmentosa GTPase regulator gene *(Rpgr*^{delEx4}*)*.

Methods: The *Rpgr*^{delEx4} mutation was bred into two mouse lines (C57BL/6 and BALB/c) over several generations. Electroretinogram was taken at different time points under scotopic and photopic conditions. For morphological characterization, thickness of the ONL, IS and OS was determined and the number of rod and cone nuclei was counted on epon sections. Immunohistochemical localization of proteins of the phototransduction cascade (rhodopsin, cone opsin, transducin and arrestin) was performed in mice at 1 month of age.

Results: The two mutant mouse strains revealed different retinal degeneration phenotypes. C57BL/6 mutant male mice displayed a progressive decrease of the rod photoreceptor amplitudes starting at the age of 3 months. Alterations in the photopic responses were only marginally. In contrast, the degenerative processes in the BALB/c background started as early as 1 month of age. The cone b-wave as well as the rod a-wave responses were found to be significantly reduced. Morphometric analyses indicated a loss of photoreceptor cells in the C57BL/6 line. Interestingly, a loss specifically of cone cells was identified in the BALB/c line. Rhodopsin and cone opsin were mislocalized in mice of both strains at 1 month of age, which suggests a defect in protein transport. Of note, localization and translocation of arrestin and transducin were not affected.

Conclusion: Both *Rpgr* mutant mouse lines developed different retinal degenerations. Our results indicate an effect of the genetic background on disease onset and progression. These findings emphasize a role for *Rpgr* in rods and in cones, which is in accordance with retinal phenotypes observed in patients with *RPGR* mutations. Additionally, early mislocalization of rhodopsin and cone opsin may indicate a role of RPGR in transport pathways. The established mouse lines provide valuable models to study RPGR function in rods and cones and the influence of genetic modifiers on the disease course.

Mechanisms of Cone Degeneration in LRAT and RPE65 knockout mice

Wolfgang Baehr, Ph.D.
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Lrat$^{-/-}$ and *Rpe65*$^{-/-}$ mice, models of human Leber Congenital Amaurosis (LCA), fail to generate 11-*cis*-retinal due to disruption of the retinoid cycle. The typical retina phenotype presents with rapid sectorial cone degeneration. S-opsin and M/L-opsin fail to traffic to cone outer segments appropriately. Concomitantly, guanylate cyclase 1, cone Tα-subunit, cone PDE6α' and GRK1 (opsin kinase) are not transported to cone outer segments. Aberrant localization of these membrane-associated proteins was evident at postnatal day 15, before the onset of ventral and central cone degeneration. Protein levels of cone Tα and cone PDE6α' were reduced, whereas their transcript levels were unchanged suggesting posttranslational down-regulation. In an *Rpe65*$^{-/-}$*Rho*$^{-/-}$ double knockout model, trafficking of cone pigments and membrane-associated cone phototransduction polypeptides to the outer segments proceeded normally upon 11-*cis*-retinal administration. These results suggest that ventral and central cone opsins must be regenerated with 11-*cis*-retinal to permit transport to the outer segments. Furthermore, the presence of 11-*cis*-retinal is essential for proper transport of several membrane-associated cone phototransduction polypeptides in these cones.

The cone dominated zebrafish retina as a model for retinal diseases

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Purpose: To show that the zebrafish retina is a suitable vertebrate model system to study cone function and dysfunction. In this presentation we want to outline the advantages of the zebrafish system by presenting a nyctalopin (nyx) knockdown fish for the study of nyctalopin function in a cone-dominant animal model.

Methods: We isolated the zebrafish nyx ortholog and designed anti-nyx morpholino oligonucleotides to disrupt nyctalopin function in zebrafish larva. Furthermore, we raised a polyclonal antibody against the protein and used immunocytochemistry to localize zebrafish Nyx. Finally, we used the optokinetic response (OKR), as well as standard electroretinograms (ERGs) to analyze retinal functionality in nyx morphants.

Results: In the zebrafish retina, nyctalopin is postsynaptically localized in both synaptic layers. Functional disruption via morpholino antisense injection leads to characteristic defects in the electroretinogram and defects in visual contrast sensitivity as assessed by OKR measurements.

Conclusion: This talk will show that the zebrafish retina is indeed a suitable model to study cone morphology and function in healthy and diseased retinas by depicting the similar role of nyctalopin in synaptic function of a cone dominated retina.

Cone, cone-rod and macular dystrophy phenotypes associated with GCAP1 mutations

Michel Michaelides, Graham E. Holder, David M. Hunt, Andrew Stockman, Caterina Ripamonti, Prateek Buch and Anthony T. Moore

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Purpose: To describe the underlying molecular genetic basis of a retinal dystrophy identified in a four-generation family and to examine the phenotype and the degree of intra-familial variability.

Methods: Detailed ophthalmological examination, colour fundus photography, autofluorescence imaging, electrophysiological testing and psychophysical assessment were performed. Mutation screening of *GUCA1A*, the gene encoding guanylate cyclase activating protein-1 (GCAP1), was undertaken.

Results: All affected subjects complained of mild photophobia and reduced central and colour vision. Onset was between the third and fifth decade, with subsequent gradual deterioration of visual acuity and colour vision. Visual acuity ranged between 6/9 and counting fingers. Colour vision was markedly reduced along all three colour axes with evidence of progressive loss with age. A range of macular appearances were seen, varying from mild retinal pigment epithelial (RPE) disturbance to extensive atrophy. Electrophysiological testing revealed a range of electrophysiological abnormalities: isolated cone ERG abnormalities, reduced cone and rod responses (with cone loss greater than rod), and isolated macular dysfunction.
The 4 coding exons of *GUCA1A* were screened for mutations in affected and unaffected family members. A single transition, A319G, causing a non-conservative missense substitution, Tyr99Cys, segregated uniquely in all affected subjects.

Conclusion: The Tyr99Cys *GUCA1A* mutation has been previously shown to cause autosomal dominant progressive cone dystrophy. This study demonstrates that this mutation can also be associated with both cone-rod dystrophy and isolated macular dysfunction. The phenotypic variation described here exemplifies the intra-familial heterogeneity of retinal dysfunction that can be observed in persons harbouring the same mutation and chromosomal segment.

A novel mutation in non-receptor tyrosine kinase ligand, PITPNM3 (Nir1) causes cone dystrophy (CORD5) in patients of Swedish origin

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Purpose: Autosomal dominant cone dystrophy (CORD5) (MIM 600977) is a rare disease predominantly affecting cone photoreceptors. The CORD5 locus was initially mapped to a 27 cM region on chromosome 17p12-p13; however the gene causing the disease was not identified. The aim of the study was identification of the genetic defect and characterization of underlying molecular mechanisms in CORD5 patients of Swedish origin.

Methods: DNA from 88 individuals from two multi-generation Swedish families originating from the same geographical area in Northern Sweden were assayed by linkage and haplotype analyses. Mutation detection was performed by PCR-RFLP and sequencing. To evaluate significance of the mutation we used web-based programs: SNPper – aminoacid variation, SIFT (Sorting Intolerant from Tolerant and PolyPhen (*Poly*morphism *Pheno*typing) (http://snpper.chip.org/bio/show-amino; http://coot.embl.de/PolyPhen/; http://blocks.fhcrc.org/sift-bin).

Results: Clinical examination of our patients demonstrated impaired function of cone cells and normal response of rods. We refined the CORD5 locus from 27 cM to 14.3 cM and identified a missense mutation, p.Q626H in the phosphatidylinositol transfer membrane-associated protein (PITPNM3) (MIM 608921). The p.Q626H mutation was absent on 322 control chromosomes of ethnically matched healthy individuals and in 140 individuals affected with autosomal dominant or recessive forms of retinitis pigmentosa. Further mutation analysis of *PITPNM3* gene in cone dystrophy patients from Germany, UK and USA resulted in modest findings.

Conclusion: The study adds one more gene on 17p, which causes retinal degeneration. We provide evidence that CORD5 in Swedish patients is a distinct clinical entity. The Q626H mutation causing CORD5 is located in the C-terminal region of the PITPNM3 interacting with a member of non-receptor protein tyrosine kinases, PYK2. Our findings of the mutations in the human homolog of *Drosophila* retinal degeneration B might indicate novel pathways and a potential important role of the PITPNM3 in mammalian phototransduction.
Determination of general importance of PITPNM3 in the development of other CORDs requires further screening for additional mutations in both familiar and isolated cases and functional studies of the recombinant mutant proteins.

Retinoschisin deficiency in macular degeneration

Thomas Loenhardt
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X-linked juvenile retinoschisis (XLRS) (OMIM #312700) was first described over 100 years ago, and since then is known to be one of the more frequent hereditary retinal disorders affecting macular function in males. A hallmark of the disease is the localized splitting of the central retina. In most patients, visual acuity is reduced to 20/100 although this can vary greatly. Thus far, treatment of XLRS has been limited to the prescription of low-vision aids. Surgical interventions benefit the patient only in rare cases.
RS1 is the causative gene associated with XLRS and encodes a retina-specific 24 kDa protein, named retinoschisin. It is secreted mainly from photoreceptors as a disulfide-linked homo-oligomeric complex that is primarily localized to the outer surface of the inner segments of cones and rods but is also seen in the outer nuclear and outer plexiform layers and the bipolar cells of the inner nuclear layer. The mature protein consists almost exclusively of a discoidin domain, a highly conserved module that likely mediates cell-cell interaction. The biological role of the discoidin domain depends on its interaction with various molecules, including growth factors, phospholipids and lipids, galactose or its derivatives, and collagen. The spectrum of *RS1* mutations comprises mostly missense mutations which have been shown to lead to misfolded, aggregated proteins mostly retained in the endoplasmatic reticulum. These findings suggest that lack of functional retinoschisin underlies disease pathology. Our studies initially focussed on the binding properties of retinoschisin to the outer membrane surface via phospholipids or collagens. In addition, retinal membranes from retinoschisin-deficient mice were assayed for binding to recombinant RS1 protein. Following this approach competitive peptide-binding assays were conducted facilitating the delineation of retinoschisin-specific epitopes in membrane binding. Our data suggested that RS1 binds exclusively to retinal membranes via a so far unknown mechanism. A recent study suggests RS1 binding to a complex consisting of Na/K ATPase and the sterile alpha and TIR motif-containing protein, SARM1. This complex may be part of a novel signalling pathway which is important for the structure and function of the photoreceptor-bipolar synapse and the normal interaction of these cells with the extracellular matrix.

Best macular dystrophy: Mechanisms of Ca2+ regulation

Sarka Krejcova[1], Vladimir M. Milenkovic[2], Olaf Strauss[2]
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Purpose: The VMD2 (Vitelliform Macular Dystrophy, Type 2) gene product, bestrophin-1, functions as Ca^{2+}-dependent Cl channel but could also influence the activity of voltage-dependent L-type Ca^{2+} channels. To identify the molecular basis of the Ca^{2+} channel regulation, immunoprecipitation experiments with heterologeous expressed Ca^{2+} channel subunits and bestrophin-1 were carried out.
Methods: In order to study a possible direct interaction between bestrophin-1 and voltage-dependent Ca^{2+} channels an approach of immunoprecipiation of heterologeously expressed proteins was used. In addition quantitative colocalization analysis has been employed to quantify the degree of colocalization of heterologeously expressed proteins.
Results: The pore-forming $Ca_v1.3$ subunits showed co-immunoprecipitation with the accessory ,3-subunits, a known physiological interaction. In co-transfection with bestrophin-1 no interaction was detected with $Ca_v1.3$. In contrast, positive interaction with β3-subunits was detected by co-precipitation. The co-immunoprecipitation between β3-Ca^{2+} channel subunits and bestrophin-1 was confirmed in freshly isolated retinal pigment epithelial cells. The detection of protein-protein interaction with β3-subunits and bestrophin-1 were independent from cell lines used as expression system (CHO, COS-7, HEK-293). Bestrophin-1 lacking proline-rich motifs on their C-terminus did not co-precipitate with β3-subunits. Using confocal microscopy interaction between wild-type bestrophin-1 and β3-subunits was detected in intact cells whereas bestrophin-1 which lacks proline-rich motifs did not show interaction with β3-subunits in intact cells.
Conclusion: Bestrophin-1 Cl channels regulate activity of voltage-dependent Ca^{2+} channels by physical interaction of bestrophin C-terminus with SH3 domains of Ca^{2+} channel β-subunits.

CRB1 viral gene therapy to prevent onset of Leber congenital amaurosis and retinitis pigmentosa.

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Purpose: Mutations in the *CRB1* gene are responsible for about 11% of Leber congenital amaurosis and 5% of autosomal recessive retinitis pigmentosa. The aim of our research is to understand retinal degeneration due to *CRB1* mutations and to develop *CRB1* viral gene therapy using the *Crb1* knockout mouse as test model.

Methods: We study the retinal phenotype in *Crb1* mutant mice and retinal explants by scanning-laser-ophthalmoscopy (SLO), immunohistochemistry, immuno-electron microscopy, gene overexpression and silencing studies. In addition, we study the function and composition of the Crb1 protein complex and develop adeno-associated viral (AAV) *CRB1* gene therapy vectors and tools to rescue the retinal phenotype.

Results: Crb1-/- mice develop retinal degeneration in the inferior temporal quadrant at 3-18 months of age. Crb1 is required to maintain adherens junctions between Müller glia cells and photoreceptors, preventing delamination of the photoreceptor layer and death of retinal neurons. Retinal degeneration is increased more than 5-fold after exposure for 3 days to 3000 lux of white light. Crb1-/- mice born in the dark also develop retinal degeneration at 6-months of age indicating that light accelerates, not initiates, retinal degeneration. CRB1 proteins in mouse and human localize to a subapical region (SAR) adjacent to adherens junctions at the outer limiting membrane. The full length Crb1 protein containing a transmembrane and 37 amino acid intracellular C-terminus is localized specifically in Müller cells, whereas photoreceptors might secrete an extracellular Crb1 form lacking the transmembrane and intracellular domain. Family members Crb2 and Crb3 localize at the SAR of Müller cells and photoreceptors. The Crb1-interacting protein Pals1/Mpp5 is required for correct localization of Crb1-3, multiple PDZ protein Mupp1, and Veli3. Expression of recombinant CRB1 by an AAV *CRB1* gene therapy vector in Müller cells of Crb1 deficient mice results in correct localization at the SAR. Some serotypes of AAV (e.g. AAV6) are capable of transducing Müller cells.

Conclusion: Expression of human CRB1 in Müller cells of Crb1 deficient retinas leads to correct localization at a subapical region immediately adjacent to adherens junctions at the outer limiting membrane. Some AAV serotypes are capable of Müller cell transduction after subretinal injection.

Fundus autofluorescence and near-infrared autofluorescence in cone and macular dystrophies

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Infrared imaging has been suggested for the evaluation of retinal dystrophies as early as in 1970. Several fluorophors are present in the retinal pigment epithelium (RPE), the two most important are lipofuscin and melanin. With a confocal laser scanning ophthalmoscope it is possible to evaluate the distribution of these fluorophors. Laser light of 488 nm excites the fluorescence of lipofuscin and recording the emisson above 500 nm shows the distribution of fundus autofluorescence (FAF), whereas laser light of 788 nm excites the fluorescence of melanin and recording the emisson above 800 nm shows the distribution of near-infrared fluorescence (NIA). These are the normal filter settings for fluorescein angiography and indocyanin green angiography.
For FAF typical patterns could be shown already for several retinal diseases.
To compare patterns changes in FAF and NIA in hereditary retinal diseases, FAF and NIA were obtained in 31 patients with hereditary cone and macular dystrophies (Stargardt disease (n=14), macular dystrophy (5), cone-rod dystrophy (12)) with the HRA 2 scanning laser ophthalmoscope (Heidelberg Engineering, Germany).
The patient's images were compared with images obtained from a series of healthy subjects. FAF and NIA images were compared for intensity of autofluorescence, lesion size and lesion pattern.
In all 31 cases FAF and NIA images showed pathological changes compared to the normal group. FAF and NIA changes presented with different patterns. In 70.9% lesion size was larger in NIA compared to FAF, in 22.6 % lesions had the same size and in 6.5% lesions were smaller in NIA. In Stargardt disease and cone-rod dystrophy specific NIA patterns could be divided in a central and more peripheral type. Mapping the presence or absence of melanin and lipofuscin in the different areas of the RPE can improve understanding of the pathophysiological changes in retinal dystrophies.

New insight into ARMS2-associated AMD pathology

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Age-related macular degeneration (AMD), a disease of multifactorial nature, is a leading cause of legal blindness in the Western hemisphere. Late stages of the disease present with atrophy of the photoreceptors and the retinal pigment epithelium (RPE) ("dry" AMD) or manifest as choroidal neovascularisation with subsequent local bleedings ("wet" AMD).

Genetic variants at two chromosomal loci, 1q31 and 10q26, confer a major disease risk, together likely accounting for over 50% of AMD pathology. Chromosome 1q31 association is tightly linked to complement factor H (CFH) suggesting an important role of inflammation and the alternative complement pathway in AMD pathogenesis. In contrast, signals at 10q26 center over two nearby genes, LOC387715/ARMS2 *(age-related maculopathy susceptibility 2)* and HTRA1 *(high-temperature requirement factor A1)*, suggesting two equally probable candidates to represent the second major AMD susceptibility gene.

Several variants with conceivable functional relevance were found within both gene loci at 10q26 demonstrating a similar extend of association with AMD. Differentiation of the signals and determination of the most likely functional variant is, however, not feasible on statistical grounds due to a high linkage disequilibrium in the region. Therefore, only the analysis of functional consequences associated with the polymorphic variants in the two genes will allow distinguishing between the two candidates.

Systemic Complement Activation in Age-Related Macular Degeneration

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Background: Dysregulation of the alternative pathway of complement (APC) cascade has been implicated in the pathogenesis of age-related macular degeneration (AMD), the leading cause of blindness in the elderly.

Methods: Parameters of systemic APC activation in blood plasma together with disease-associated genetic markers in AMD patients were determined. Plasma concentrations of complement activation products C3d, Ba, C3a, C5a, SC5b-9, substrate proteins C3, C4, factor B and APC regulators factor H and factor D were quantified in patients (n=112) and controls (n=67). Subjects were analyzed for single nucleotide polymorphisms in factor H *(CFH)*, factor B-complement component C2 gene *(BF-C2)* and complement C3 *(C3)* genes which were previously found to be associated with AMD.

Results: All complement activation products, especially markers of chronic APC activation Ba and C3d ($p < 0.001$), were significantly elevated in AMD patients compared to controls. Similar alterations were observed in factor D, but not in C3, C4 or factor H. Logistic regression analysis revealed that a model that is based only on APC activation markers Ba, C3d and factor D has considerably better discriminative ability to identify AMD patients within our study population compared to a model based on known genetic markers of the complement system. In both the controls' and AMD patients' group, the protein markers of APC activation were correlated with *CFH* haplotypes.

Conclusion: This study is the first to show systemic complement activation in AMD patients and its association with genetic variants of *CFH* which were previously linked to AMD.

POSTER ABSTRACTS

RETINAL CELLS INTEGRATE INTO THE OUTER NUCLEAR LAYER AND DIFFERENTIATE INTO MATURE PHOTORECEPTORS AFTER SUBRETINAL TRANSPLANTATION INTO ADULT MICE

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Vision impairment caused by degeneration of photoreceptors, termed retinitis pigmentosa, is a debilitating condition with no cure presently available. Cell-based therapeutic approaches represent one treatment option by replacing degenerating or lost photoreceptors. In this study the potential of transplanted primary retinal cells isolated from neonatal mice to integrate into the outer nuclear layer (ONL) of adult mice and to differentiate into mature photoreceptors was evaluated. Retinal cells were isolated from retinas of transgenic mice ubiquitously expressing enhanced green fluorescence protein (EGFP) at either postnatal day (P) 0, P1 or P4 and transplanted into the subretinal space of adult wild-type mice. One week to 11 months post-transplantation experimental retinas were analyzed for integration and differentiation of donor cells. Subsequent to transplantation some postnatal retinal cells integrated into the ONL of the host and differentiated into mature photoreceptors containing inner and outer segments as confirmed by immunohistochemistry and electron microscopy. Notably, the appearance of EGFP-positive photoreceptors was not the result of fusion between donor cells and endogenous photoreceptors. Retinal cells isolated at P4 showed a significant increase in their capacity to integrate into the ONL and to differentiate into mature photoreceptors when compared with cells isolated at P0 or P1. As cell suspensions isolated at P4 are enriched in cells committed towards a rod photoreceptor cell fate it is tempting to speculate that immature photoreceptors may have the highest integration and differentiation potential and thus may present a promising cell type to develop cell replacement strategies for diseases involving rod photoreceptor loss.

HISTONE DEACETYLASE ACTIVITY IN THE RETINA OF THE RD1-MOUSE

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Purpose: Histone acetylation/deacetylation alters the chromatin structure activating or repressing transcription, and the deacetylation step is performed by the Trichostatin A (TSA) sensitive histone deacetylases Class I and II (HDACs) and the TSA insensitive Class III HDACs (Sirtuins). In addition to a role in transcriptional regulation, the Sirtuins are also found to be important for life span extension and therefore have been proposed to play a neuroprotective role. Since various retinal degenerations can be looked at as nothing else but a shortened life span of neuronal cells, our aim was to establish an HDAC-assay that could be performed on retinal sections, thus assessing the various HDAC-activities in defined cells from either normal or degenerating retinas.

Methods: Retinas from mice with inherited retinal photoreceptor degeneration (rd1 mouse) or from wild-type counterparts were cryosectioned and assessed for deacetylation activity. The latter used a fluorescence based technique, that enabled us to use fluorescence microscopy to monitor the deacetylation of a given substrate over time.

Results: The K_m-values for the general HDAC activity as well as the Sirtuin activity in the whole retina were determined, which gave values that were in the same order of magnitude as those described in the literature. Furthermore, we could show that the total HDAC activity was the sum of the activity of the Class I/II HDACs and that of the Sirtuins. Although the intracellular HDAC activity was monitored indirectly, in the sense that the deacetylated substrate carrying the read-out signal diffused out of/away from the cells after the deacetylation had taken place, we consider our results as proof-of-principle for the technique. Importantly, with extended incubation time and an additional fixation step, we observed that several cells in the photoreceptor layer of the rd1-mouse retina showed a strong HDAC-activity that was absent in the wild-type controls. This HDAC-activity could be assigned to an increased activity of the Class I/II HDACs, because it could be suppressed by Trichostatin A, whereas nicotinamide, a Sirtuin inhibitor, was ineffective in this respect.

Conclusions: HDAC activity could be measured in situ in retinal sections and a correlation between Class I/II HDAC acitivity and retinal degeneration was observed. Future experiments will show if this is a cause or a consequence of the degeneration.

OPSIN EXPRESSION IN THE DEVELOPING RAT RETINA IN VIVO AND IN VITRO.

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Purpose: The aim of present study was to establish how opsin expression proceeds in the developing retinal network *in vivo* and *in vitro* and to answer whether it is possible to develop M-cones in a rodent organotypic culture.

Methods: For the *in vivo* studies, retinas of Brown Norway rats were collected at different developmental ages (P0, P2, P4, P6, P8, P10, P12, P14, P18, P30, P60, P120 and P270). For the *in vitro* studies, retinas were prepared from P0-P2 animals as described elsewhere (Pinzón-Duarte et al. 2000, Vis. Res. 40, 3455-65), and allowed to develop in organ culture for 2 to 15 days. After fixation, both *in vivo* and cultured retinas were examined by conventional histological techniques and immunohistochemistry using specific antibodies for SWS and MWS opsins (JH455 and JH492 respectively; gift from Dr. J. Nathans). Morphometric analyses were performed on retinal whole mounts using confocal microscopy.

Results: S- and M-cones were uniformly distributed over the retina in all examined stages. S-cones were already present at birth whereas M-cones were only detected after P4. S-cones reached their maximal density at P10 (~14600 cones/mm2), M-cones at P12 (~12500 cones/mm2). Thereafter, the number of cones decreased gradually the values of about 980 S-cones/mm2 and 6450 M-cones/mm2 at P60. Final values at P270 were 950 S-cones/mm2 and 4600 M-cones/mm2. *In vitro* the immunostaining pattern was similar to that observed in vivo, but the density of labeled cones was under the *in vivo* values at any investigated age.

Conclusions: In this study we present an alternative culture method of the postnatal rat retina that allows the M-cone development independently from the time of explantation. Other than in organotypic cultures of the mouse retina we show that it is possible to study the development of both cone photoreceptor phenotypes in the rat retina in organ culture and that S- and M-opsin immunoreactivites are comparables to that of their age-matched counterparts *in vivo*. Thus our culture system is suitable for the identification and investigation of factors crucial in regulating opsin expression and cone patterning during the development of the mammalian retina.

FUNCTIONAL ANALYSIS OF MUTANT GCAP1 PROTEINS INVOLVED IN HEREDITARY CONE DYSTROPHIES

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Guanylate cyclase-activating protein 1 is expressed in rods and cones of the vertebrate retina and operates as an important Ca^{2+}-sensor in these cells. It controls the activity of membrane bound guanylate cyclases (GCs) in a Ca^{2+}-dependent manner. These steps are part of negative feedback loops in the photoreceptor cell involved in light adaptation. A mutation screening on patients suffering from autosomal dominantly inherited cone or cone rod dystrophy led to the identification of one known and three novel single point mutations in the GUCA1A gene (GCAP1, guanylate cyclase activating protein 1). These are L151F (Jiang et al. Mol. Vis. 2005; 11:143-51) and E89K, D100E and G159V. In order to analyze the functional consequences of the mutations on the protein level, corresponding expression constructs were generated, expressed heterologously in *E.coli* and purified for further studies. Mutants were tested for their ability to activate GC using bovine rod outer segment membranes as an assay model system. Ca^{2+}-dependent dose-response curves were shifted in all cases to higher values of free [Ca^{2+}]. A dramatic shift was observed with the mutant D100E indicating that under physiological conditions GC would be permanently active. However, a first test, whether this shift is due to a distortion of Ca^{2+}-induced conformational changes was negative, since intrinsic tryptophan fluorescence and electrophoretic mobility shifts were very similar for mutants and wildtype. Our data indicate that the mutations could disturb the Ca^{2+}-homeostasis in cone (and rod) cells and thereby trigger apoptotic pathways.

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DELETION OF CFHR1 AND CFHR3 PROTEINS IN AGE-RELATED MACULAR DEGENERATION

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Purpose: Age-related macular degeneration (AMD) is the most common cause of blindness in the Western World with approximately 2.5 Mio affected people in Germany. Recent studies show that several variants of human complement genes increase or decrease the individually risk for this complex eye disease. Thus, a common haplotype resulting in the large chromosomal deletion of the two "Complement Factor-H-Related" genes CFHR1 and CFHR3 results in a lower risk for AMD. This scenario correlates with the absence of the corresponding proteins in human plasma.

Methods: We examined a German AMD group of 112 patients with clinical documented AMD and 67 unaffected age-matched controls for the presence of CFHR1, CFHR2, CFHR3 and CFH, in sera. By western blot analyses with specific antibodies we defined three separated groups: homozygous for the CFHR1/CFHR3 deletion, heterozygotes and homozygotes for the two alleles.

Results: Homozygous CFHR1/CFHR3 deletion was significantly less frequent in AMD-cases than in controls. Also heterozygosity for the deletion showed a protective effect in the control group. The CFHR2 protein was equally present in the AMD- and the control group.

Conclusion: Within this first national AMD cohort we demonstrate that a lack of the CFHR1 and CFHR3 proteins in human plasma occure at a lower frequency in AMD patients, thus confirming former results for a German cohort. Moreover we define a heterozygous scenario and analyzed, with CFHR2, a further protein of the complement cascade and its influence on the development of AMD.

INTRAVITREAL ANTI-VEGF THERAPY IN TYPE 2 IDIOPATHIC MACULAR TELANGIECTASIA: LONG-TERM FOLLOW-UP

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Purpose: Type 2 idiopathic macular telangiectasia (type 2 IMT) is a rare bilateral macular disease which causes neurosensory macular atrophy. Deterioration of visual acuity and the development of parafoveal scotomas typically occur during the 5th to 7th decade. A genetic predisposition has been suggested due to the occurrence in families and monozygotic twins. So far, therapy has been very limited. However, vascular endothelial growth factor (VEGF) has recently been implicated to play a role in the pathogenesis of the disease and short-term results of therapeutic intervention by intravitreally injected anti-VEGF drugs were promising.

Methods: Six eyes of five patients with type 2 IMT received two doses of intravitreal bevacizumab (1.5mg) at a four week interval, followed by further applications depending on disease activity. Examinations included biomicroscopy, standardized visual acuity (VA) testing, fluorescein angiography, retinal thickness analysis by optical coherence tomography and fundus-controlled microperimetry.

Results: Mean follow-up time was 18 months (range 16-21 months). Mean VA at four selected time points (one month after second treatment, one month and three to four months after last treatment, and at last visit) increased significantly (by 8.8, 6.3, 7.7, and 8.7 ETDRS letters, respectively; all $p \leq 0.05$). Parafoveal ectatic capillaries and leakage in fluorescein angiography as well as mean central retinal thickness decreased in all eyes following treatment. A rebound effect was observed after 3-4 months and at the last visit, retinal thickness was increased in selected retinal sectors including the fellow eye.

Conclusion: Inhibition of vascular endothelial growth factor (VEGF) by intravitreally injected bevacizumab may lead to functional improvement as well as transient decrease of leakage and retinal thickness in patients with type 2 IMT. The data suggest that there is a VEGF-mediated "active" disease stage in which treatment may be most effective.

ANALYSIS OF IN VIVO PRENYLATION OF RABGTPASES: EVIDENCE OF A PRENYLATION HIERARCHY WITH RELEVANCE TO CHOROIDEREMIA?

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Protein prenylation is a post-translational form of lipid modification in which geranylgeranyl or farnesyl groups are conjugated to the C-terminal cysteins of a variety of proteins. The most commonly prenylated proteins are the geranylgeranylated Rabs, required for the regulation of vesicle trafficking.
In order to be prenylated, Rab proteins are bound by the Rab escort protein (REP) and presented to the Rab Geranylgeranyl transferase (RabGGTase), which prenylates the Rab on (usually) two C-terminal cysteins. REP then binds the insoluble geranylgeranyl groups, disengages from RabGGTase and delivers the Rab to the appropriate membrane where it can undergo GDP/GTP exchange and carry out its roles in membrane trafficking.
Defects in prenylation can result in vesicle transport pathologies such as Choroideremia or Hermansky-Pudlak syndrome.
Choroideremia is an X linked disease, characterised by the degradation of retinal cells and blindness by middle age. It is caused by the mutational knock out of REP1. REP2 can almost compensate however, large amounts of unprenylated Rab27A build up in the lymphoblasts of choroideremia patients. The connection between the loss of REP1 and retinal degeneration is not known.
The dissociation constant for the REP2:Rab27A interaction is four fold higher than for the REP1:Rab27A interaction. However, as this is comparable to a 2.7 fold difference for Rab1 and a 6 fold difference for Rab7, it does not explain the build up of unprenylated Rab27A. In absolute numbers however, the dissociation constant for Rab27A (with either REP1 or REP2) is four fold higher than for Rab1 and over 150 fold higher than for Rab7. The difference in dissociation constants for different Rabs may establish a prenylation hierarchy. Therefore if total REP activity becomes limiting Rab27A will be unable to compete favourably with other Rabs for prenylation.

We have used two methods to study prenylation *in vivo*.
Microinjection of fluorescently labelled Rabs, or of Rabs with prenylation machinery, or of in vitro prenylated Rabs, shows, by a membrane bound versus cytosolic distribution, how quickly Rabs are prenylated. This has provided evidence that a prenylation hierarchy does exist and that Rab27A is indeed prenylated at a significantly slower rate than either Rab7 or Rab1.
Secondly, an *in vitro* prenylation assay can label unprenylated Rabs with a Biotin tagged ana-

logue of geranylgeranyl (B-Gpp). The assay is sensitive enough to identify unprenylated Rab27A in choroideremic cells moreover, the biotin tagged Rabs can be concentrated using streptavidin bead pull-down technology. This assay will be an essential tool to investigate the importance of REP1 versus REP2 in choroideremia
Now we plan to transfect choroideremic cells with GFP tagged REP1 and REP2, or even RabGGTase, followed by FACS sorting and analysis of the Rab prenylation status of sub-populations, to measure whether over expression of the prenylation machinery can rescue underprenylation.

DEFECTS IN SPLICING AS A POSSIBLE CAUSE OF RETINITIS PIGMENTOSA

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The majority of Retinitis Pigmentosa (RP) relevant gene loci encode for proteins with roles in the visual process. However, RP can also be caused by mutations in the pre-mRNA splice factors hPrp3, hPrp31 and hPrp8. All these factors are integral components of one functional subunit of the spliceosome, the so-called tri-snRNP particle. Therefore, a key question for the molecular understanding of RP is how the weakening of splicing, a general cellular pathway, translates into a tissue specific phenotype.

Purpose: We have characterized the biochemical effects of RP mutations in splicing factors and screened for RP-linked mutations in other splice factor genes. In addition we have begun to analyze RP-associated splice factors in the zebrafish model system.

Methods: Biochemical approaches are being used to examine, whether splicing factors carrying pathogenic mutations exhibit defects in their interaction with other cellular partners. To analyze the consequences of reduced expression of RP associated splice factors as well as to functionally characterize RP mutants, a morpholino knock down strategy and gene overexpression is used in zebrafish as model organism.

Results: We have previously reported a RP patient that carries a missense mutation in the tri-snRNP component hPrp4. A detailed biochemical analysis revealed impaired association of this mutant with the tri-snRNP. This is caused by its failure to bind to hPrp3, a well known interactor of hPrp4. Severe knock down of the hPrp4 zebrafish homologue zfPrp4 leads to drastic developmental defects that can be rescued by the wild type protein, but not pathogenic zfPrp4 mutant. Analysis of morphant retinae by immunocytochemistry showed that a mild knock down of zfPrp4 leads to morphological defects of the photoreceptor cell layer. A very similar phenotype was observed upon knock down of zfPrp31, another RP related splice factor.

Conclusion:
Our biochemical investigation revealed defects of mutant hPrp4 found in a RP patient to form

the tri-snRNP particle of the spliceosome. Furthermore reduced zfPrp levels in zebrafish cause specific photoreceptor defects. Together our data are consistent with the idea that reduced splicing leads to Retinitis Pigmentosa in patients with mutant hPrps. We speculate that this biochemical defect affects processing of certain pre-mRNA transcripts relevant for the retina and hence lead to tissue specific phenotype of RP.

ELUCIDATION OF THE GENETIC BASIS OF USHER SYNDROME IN CANADIANS OF FRENCH ORIGIN: IMPLICATIONS FOR DIAGNOSTIC AND CLINICAL MANAGEMENT

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Purpose: To genetically characterize USH1 and USH2 patients from the Canadian provinces of Quebec and New Brunswick (former Acadia).

Methods:
USH1: We performed genotyping of polymorphic microsatellite markers for the seven USH1 loci in parallel with a mutation screening strategy in 15 USH1 patients. Once a mutation was identified, all patients were screened for it. Where this did not lead to the identification of the genetic subtype, we sequenced the entire coding regions of all USH1 genes. Haplotype analysis was performed for the USH1C locus.
USH2: Patients (n = 9) were screened by direct sequencing for mutations in the three known USH2 genes (USH2A, GPR98 (USH2C) and DFNB31 (USH2D)). Haplotype analysis was performed for the USH2A locus.

Results:
USH1: 60% of cases were due to mutation of the USH1C gene. The founder mutation c.216G>A represents 40% of disease alleles in Quebec and has a carrier frequency in the general population of 0.44% (1/227). c.216G>A has previously been designated the "Acadian allele" because it accounts for virtually all Acadian cases. Haplotype analyses revealed a common c.216G>A-associated haplotype in all patients from Quebec and in an Acadian USH1C patient.
USH2: USH2A mutations were identified in eight patients (88.9%) The founder mutation c.4438_4439delCT in the USH2A gene accounts for 10/18 disease alleles (55.6%). All patients carried the same c.4438_4439delCT–associated haplotype. c.4338_4339delCT has previously been described in the literature in two Acadian patients and was found in homozygous state in all Acadian families of our sample. One male patient carried mutations in the GPR98 gene (USH2C).

Conclusions: Founder mutations in the USH1C and USH2A gene underlie Usher syndrome type 1 and 2 in Quebec, respectively. For c.216G>A (USH1C) and c.4438_4439delCT (USH2A), patients from Quebec and former Acadia carry the identical respective haplotypes. So far, Acadians and French Canadians – even though both populations descend from French settlers - have always been thought to be genetically distinct. Our findings refute this common assumption and show that early settlers must have contributed to the gene pool of both populations. With a limited number of molecular tests, it will now be possible in these populations to estimate whether a congenitally hearing-impaired child will develop additional retinal disease in later life.

Reference: Ebermann et al. (Genome Biol-Zitat)

A COMPARISON OF NEURONAL CELL DIFFERENTIATION IN VITRO WITH DENTAL FOLLICLE PRECURSOR CELLS AND RETINAL PROGENITOR CELLS

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Background: Although different cell types in the adult retina are suggested as putative retinal stem cells, the human retina does not demonstrate any sign of spontaneous regeneration. The loss of retinal cells is therefore generally regarded to be the irreversible lost of vision. A strategy to restore vision after retinal degeneration is a cell replacement therapy. This study compared the retinal cell differentiation of murine dental follicle precursor cells (mDFCs) with murine retinal progenitor cells (mRPCs) under *in vitro* conditions for the evaluation of DFCs as a new source of cells for autologous retinal transplantation.

Methods and Results: mDFCs and mRPCs were isolated from dental germs and from retinas respectively of mice. For this study a number of protocols were applied for neuronal differentiation. The success of cell differentiation was evaluated by qRT-PCR or immunocytochemical investigations for neuronal/retinal cell markers. Both mRPCs and mDFCs differentiated into cells with neuronal cell phenotypes. However, differentiated mRPCs demonstrated a more complete neuronal cell phenotype than mDFCs after neuronal differentiation. For a comparison of molecular mechanisms in mDFCs and mRPCs, the activation of signaling pathways was recorded during retinal cell differentiation. Here, remarkable differences in FGF, Hedgehog, Notch and TGFβ,/BMP signaling were detected between both cell types.

Conclusion: Dental stem cells are able to differentiate into variety of cells, offering promising approaches for stem-cell-mediated tissue regeneration. This study demonstrated that cell differentiation of mDFCs into retinal/neuronal cells is possible. However, mRPCs demonstrated a more complete differentiation into retinal cells than mDFCs. An adjustment of neuronal differentiation protocols may improve the success of retinal cell differentiation of mDFCs.

EXPRESSION OF VOLTAGE-GATED SODIUM CHANNELS IN GROWTH CONES OF THE REGENERATING RAT RETINA

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Purpose: Successful regeneration and re-establishment of synaptic connections in the adult central nervous system is a complex process determined by both the exterior environment and the endogenous neural activity of the regenerating growth cones. Voltage-gated sodium channels (Nav) are important for the correct targeting of projecting axons in the developing visual system. Here we investigated the biophysical properties of voltage-gated sodium channels in an organotypic explant culture of the adult rat retina.

Methods: The optic nerve of adult rats was crushed, and an explant culture of the retina was established 5 days post surgery. The explants were cultured with the ganglion cell side down on hydrophilic dishes coated with poly-D-lysine and laminin. Outgrowth of ganglion cell axons started as early as one day after explantation of the retina. Regenerating axons and growth cones of retinal ganglion cells were positive for GAP-43. We recorded directly from the growth cones using the whole-cell configuration of the patch-clamp technique.

Results: The growth cones displayed a high input resistance of 1.29 GΩ, and their resting membrane potential was –69 mV. All growth cones responded to depolarizing voltage steps with fast transient inward currents mediated by Na^+ ions, followed by slow and sustained outward K^+ currents. Half-maximal activation clustered in two groups with average potentials of 34.4 mV and 41.3 mV, suggesting the presence of at least two Nav channel isoforms. Steady-state inactivation was characterized by a half-maximal value of 72.9 mV, indicating that only 43 % of all Nav channels are available at the resting membrane potential. Growth cone Na^+ channels recovered from fast inactivation with a time constant of 4 ms. The density of Na_v channels at the growth cones was sufficient to trigger the generation of a single action potential. In contrast, depolarization of cell bodies of retinal ganglion cells resulted in the generation of a series of fast, all-or-none action potentials.

Conclusion: Our data provide for the first time a detailed description of the biophysical properties of Nav channels expressed in retinal growth cones of the regenerating rat retina, which is important for a thorough understanding of the role of Nav channels in regeneration and retinal plasticity.

FUNDUS AUTOFLUORESCENCE IN PATIENTS WITH PSEUDOXANTHOMA ELASTICUM

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Background: Pseudoxanthoma elasticum (PXE) is an inherited multisystem disorder of the elastic tissue. Typical ocular manifestations include angioid streaks, peau d'orange, salmon spots and choroidal neovascularization (CNV). PXE may serve as a model disease where changes in Bruch's membrane lead to progressive atrophy of the retinal pigment epithelium (RPE) and visual loss. As the RPE-photoreceptor complex can be investigated in vivo using fundus autofluorescence (FAF), a large cohort of PXE patients was investigated by FAF imaging.

Methods: Fourty-six patients (92 eyes) were investigated using digital fundus photography, fluorescein angiography (FA) and FAF imaging. The diagnosis was confirmed by multisystem clinical examination, mutation analysis of the ABCC6 gene and skin biopsy. FAF imaging, FA and ICGA were recorded using a confocal scanning laser ophthalmoscope (Heidelberg Retina Angiograph 2, HRA2; Heidelberg Engineering, Heidelberg, Germany), employing a wavelength of 488 nm for FAF imaging.

Results: Mean age of the patients' cohort was 50 years (SD 13.8, range 13-74) and mean visual acuity 20/125 (LogMAR 0.8, SD 0.69). Fundus changes typical for PXE were observed in all eyes. Angioid streaks were detected in all but two eyes. Focal spots of increased FAF alongside angioid streaks corresponded to the parastreak phenomenon. Peau d'orange was hardly detectable on FAF, whereas salmon spots were apparent. Drusen of the optic nerve head (5 eyes) and pattern dystrophies (8 eyes) were observed only in a minority of patients. RPE atrophy typically was widespread and heterogeneous, located mostly adjacent to angioid streaks or CNVs.

Conclusion: Abnormalities of the RPE-photoreceptor complex detected by FAF imaging were more diverse and widespread than expected from conventional fundus imaging. Such extensive alteration of the RPE suggests a possible role of pathological RPE changes in the evolution of visual loss in PXE. A generalized calcification of Bruch's membrane, as has been described, may lead to widespread RPE dysfunction and subsequent atrophy which is determining prognosis.

Key words: Angioid streaks, fundus autofluorescence (FAF), pseudoxanthoma elasticum, pattern dystrophy, peau d'orange, salmon spots

LINES OF INCREASED FUNDUS AUTOFLUORESCENCE IN RETINAL DYSTROPHIES: FUNCTIONAL AND MORPHOLOGICAL CORRELATES

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Purpose: To assess the functional significance of lines of increased fundus autofluorescence (FAF) associated with various retinal dystrophies by microperimetry and to describe their underlying morphological correlate by simultaneous confocal scanning laser ophthalmoscopy (cSLO) and spectral domain (SD)-OCT imaging.

Methods: Patients with macular and retinal dystrophies were assessed by retinal imaging including cSLO FAF imaging and simultaneous cSLO and SD-OCT imaging (Spectralis HRA+OCT, Heidelberg Engineering, Germany), respectively. Fundus-controlled microperimetry (MP1, Nidek Technologies, Padova, Italy) was performed to determine the impact of abnormal FAF on corresponding retinal light sensitivity.

Results: A discrete arched line of increased FAF was observed in patients with pigmented paravenous retinochoroidal dystrophy (PPRCA), sector retinitis pigmentosa (RP), typical RP, and bull's eye macular dystrophy without obvious correlate on fundus biomicroscopy. The orientation of these lines differed from ring shape in RP and bull's eye macular dystrophy, a semi-circle structure in sector RP to crescent shape with tiplike extensions towards branching retinal veins in PPRCA. Microperimetry indicated that the arc sharply delineates areas of severely impaired retinal sensitivity. Simultaneous FAF and SD-OCT imaging revealed that the line of increased FAF correlates with the junction between an area with preserved and an area with abnormal outer neurosensory retinal layers.

Conclusion: The findings indicate that arcs of increased FAF in retinal dystrophies demarcate areas with normal from areas with impaired retinal sensitivity. While the underlying pathogenetic mechanisms are unknown, it may be speculated that the increased FAF signal result from an increased metabolic load of corresponding RPE cells and subsequent excessive accumulation of fluorophores in the lysosomal compartment due to phagocytosis of components of diseased photoreceptors.

IDENTIFICATION OF ALTERED microRNA EXPRESSION PROFILE IN THE MOUSE HYPOXIC RETINA

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Purpose: MicroRNAs (miRNAs), the newly recognized regulators of gene expression, affect and control a diverse range of normal and pathological cellular processes (1).
Retinal cells exposed to hypoxia respond by increasing the stability of HIF-1a, a major hypoxic signaling protein that activates transcription of numerous genes mediating also cell survival (2). This study aims at the identification of the miRNA expression pattern in the hypoxic mouse retina and focuses on the identification of miRNAs linked to hypoxia inducible genes as potential modifiers of photoreceptor cell survival.

Methods: Adult wild-type mice were exposed to 6% O_2 for 6h. Total RNA from retinas pooled as triplicates was purified using TRIzol immediately after preconditioning or after 2 or 4h of reoxygenation. MiRNA expression profile of hypoxic and normoxic samples were analyzed on miRCURY™ LNA microRNAs Arrays (Exiqon). MiRNA targets were detected *in silico*.

Results: Altered miRNA expression patterns in the hypoxic vs. normoxic retina have been identified. Potential target genes for most differentially expressed miRNAs have been assigned.

Conclusions: The preliminary results indicate that miRNAs might be involved in the regulation of the retinal response to hypoxia. The suggested modulatory role of specific miRNAs for the expression of hypoxia responsive genes may connect microRNAs with cell survival and neuroprotection in the retina.

1. Williams AE (2007) Functional aspects of animal microRNAs. Cell Mol Life Sci 29
2. Grimm C., Wenzel A., Groszer M., Mayser, H., Seeliger, M., Samardzija, M., Bauer, C., Gassmann M., Remé C.E. (2002) HIF-1 – induced erythropoietin in the hypoxic retina protects against light-induced retinal degeneration. Nature Med. 8: 718-724

Keywords: hypoxia, microRNAs, cell survival, retina;

CHARACTERIZATION OF A ZEBRAFISH PRP8 MUTANT

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Retinitis pigmentosa (RP) is caused by mutations in genes expressed exclusively in the eye, with the constitutively expressed essential splicing factors hPrp3, hPrp31 and hPrp8 being the exception to the rule. These cases demand for a model where tissue specific effects of altered gene expression can be studied in an isogenic but generally unaffected organism, placing special interest to the development of an animal model resembling such a condition.

Purpose: To mimic the situation in patients, a zebrafish line carrying a presumptive null allele of the zebrafish Prp8 homologue (zfPrp8) has been created. This mutant will be characterized in regard to its usability as a model for retinal degeneration.

Methods: Fish carrying one mutant Prp8-allele are identified by restriction fragment length polymorphism (RFLP) analysis. Heterozygous (+/-) fish are investigated for signs of visual impairment at various stages of development by retinal immunohistochemistry and behavioral analyses. Similar assays are performed with larvae that have been injected with Morpholino inhibiting zfPrp8 expression.

Results: Heterozygous larvae can be identified by RFLP analysis despite of having no obvious phenotype. Up to four days post fertilization (dpf) no reduction of rod photoreceptors in retinal sections could be observed. However, analysis of the optokinetic reflex reveals a significant reduction in the number of saccades when compared to fishes from wildtype strain. Interestingly, analysis of the optomotoric reflex of (+/-) fish demonstrates a reduction in swimming rate at 5dpf that declines until 15dpf where they perform similar to wildtype. Initial morphological analysis of adult (+/-) fish points towards a reduced number of rod photoreceptors.

Conclusions: Complementary to Morpholino mediated knockdowns of splicing factor expression that are ideally suited for the investigation of phenotypes observed earlier in development, the generation of a zfPrp8 mutant allows examination of animals at all stages of development. While zfPrp8 (+/-) larvae show only a mild developmental phenotype, preliminary results obtained for adult (+/-) retinae indicate a rod photoreceptor degeneration phenotype that is currently under investigation.

INVESTIGATION OF ENDOPLASMIC RETICULUM (ER) STRESS IN AN SFD MOUSE MODEL

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Purpose: Sorsby fundus dystrophy (SFD) is an autosomal dominant macular degeneration caused by mutations in the gene encoding tissue inhibitor of metalloproteinase-3 (TIMP3), a multifunctional protein tightly associated with the extracellular matrix. Here, we have studied the impact of ER stress on SFD pathogenesis using an SFD mouse model carrying the SFD-related S156C mutation in the murine *Timp3* gene.

Methods: Primary fibroblasts cell lines were established from mouse lungs and used to determine the subcellular localization of wild-type and mutant TIMP3 and to prepare cell and ECM extracts for Western blot analysis. Unfolded protein response (UPR) was evaluated by quantitative real-time RT-PCR and immunoblotting using RNA and protein extracts derived from fibroblast cells and dissected RPE/choroid tissue.

Results: ECM and cell extracts of mutant fibroblasts (S156C/+ and S156C/S156C) were shown to contain significant amounts of oligomerized TIMP3. Immunocytochemistry revealed an accumulation of mutant S156C-TIMP3 protein in the ER of fibroblasts when compared to wildtype. Various UPR marker genes were shown to be activated in mutant cells.

Conclusions: Our results suggest that accumulation and misfolding of mutant S156C-TIMP3 protein may induce UPR in an SFD mouse model.

THE ROLE OF JAK-STAT SIGNALING IN RETINAL DEGENERATION

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Purpose: To determine the role of the Janus kinase - Signal Transducer and Activatior of Transcription (Jak-STAT) signaling pathway in retinal degenerations. This was investigated in mouse models if induced photoreceptor apoptosis (light exposure) and /or neuroprotection (hypoxic preconditioning).

Methods: Retinas were isolated from hypoxic preconditioned and/or light exposed wild type mice. Gene expression of several genes involved in the Jak-STAT signaling pathway was measured by semi-quantitative real time polymerase chain reaction (real time PCR).

Results: It was shown earlier that hypoxic preconditioning is neuroprotective to the retina in models of light induced photoreceptor cell damage. Here we show that Janus kinase 3 (Jak3) is similarly induced in the retina of light exposed mice which were or were not preconditioned by hypoxic exposure. In contrast, Src-homology 2 domain phosphatase-1 (shp-1) was induced only in mice which were not protected by hypoxic preconditioning.

Conclusions: Since light exposure induced Jak3 expression in protected (hypoxic preconditioned) as well as in not protected animals, Jak3 may be involved in an endogenous response pathway activated as part of a neuroprotective response. In contrast, the restricted induction of shp-1 in animals susceptible to light damage suggests that shp-1 may be an important apoptosis signal.

Keywords: Jak-STAT, retinal degeneration, apoptosis, neuroprotection

GENE EXPRESSION PROFILING OF CHONDROITIN SULFATE DISACCHARIDE STIMULATED MICROGLIA REVEALS A NOVEL ALTERNATIVELY ACTIVATED PHENOTYPE

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A disaccharide degradation product of chondrotin sulfate proteoglycan, CSPG-DS, has been previously implicated in the inhibition of neurodegeneration by influencing microglia activation. In this study, genome wide microarray analysis and real time qRT-PCR validation was used to identify specific gene expression profiles of CSPG-DS stimulated microglia. Gene products involved in phagocytosis, detoxification, migration, immune regulation and antigen presentation were found to be significantly altered. Importantly, a unique transcriptional phenotype related to alternatively activated (M2) macrophages with anti-inflammatory properties was identified. Using functional cell assays, we found that CSPG-DS stimulated microglia possess increased phagocytic capacity, but lack direct cytotoxic effects such as secretion of nitric oxide. Furthermore, CSPG-DS microglia did not diminish the viability or cause apoptosis of cultured photoreceptor cells, which were strongly affected by conditioned media of LPS and IFN-gamma treated cells. Taken together, our data provide a unique transcript dataset and important *in vitro* findings on the functional properties of CSPG-DS activated microglia. These might be starting points to explore the *in vivo* role of CSPG-DS as bioactive microglia regulator and its potential therapeutic application in immune related neurodegenerative disorders.

THE Y402H POLYMORPHISM IN CFH AND FHL 1 AFFECTS MCRP BINDING

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Purpose: Age-related macular degeneration (AMD) is the most common cause of blindness in the Western World and affects about 50 million people worldwide. Recently, the Y402H variant of complement Factor H (CFH) was shown to be strongly associated with AMD. The resulting tyrosine to histidine exchange at amino acid position 402, occurs within short consensus repeat (SCR) 7 and affects binding to heparin and CRP. This leads to functional impairment in fluid phase and on the surface of retinal pigment epithelial cells. The CFH gene encodes two proteins: CFH and the alternatively spliced product FHL 1. Both proteins are present in human plasma and both carry the Y402H polymorphism, thus resulting in inappropriate complement control.

Methods: In order to analyze the functional influences of a reduced CRP-binding for a regular complement control, we purified CFH from plasma of AMD patients (YY402 and HH402) and in addition expressed the two recombinant variants of FHL 1 (FHL 1_{Y402} and FHL 1_{H402}).
The generation of mCRP occured by heat and urea treatment.

Results: The risk variants of both: CFH-protein$_{HH402}$ and recombinant FHL 1_{H402} protein showed significantly reduced binding to the monomeric form of CRP as compared to the protective variants. Cofactor- and flow cytometry assays show that mCRP affects cofactor activity in fluid phase and recruitment of CFH and FHL 1 to the surface of necrotic cells.

Conclusions: The risk forms of CFH and FHL-1 show a reduced interaction to the ligand mCRP and to necrotic cells. This effect may lead to an inadequate complement control on the surface of host cells. Thus the decreased recruitment of the complement regulators to necrotic cells and tissues may enhance local inflammation in the eye and thus may lead to development of AMD.

SUCCESSFUL PHOTORECEPTOR TRANSPLANTATION INTO THE AGED *CCL2*/MCP-1 MOUSE MODEL OF AGE-RELATED MACULAR DEGENERATION

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Purpose: Age-related macular degeneration (AMD) is the leading cause of blindness in the developed world and leads to loss of macular photoreceptors. Photoreceptor replacement by transplantation is therefore a potential future therapeutic strategy. It is not yet known whether transplanted photoreceptors can integrate into the aged retina. Therefore, we analysed the efficiency of photoreceptor transplantation in aged compared with young adult recipient mice. The *Ccl2* knockout (MCP-1) mouse has been described as a model for age related retinal disorders with impaired macrophage recruitment. With age, these mice develop phenotypic features similar to those seen in human AMD and since macrophages may play a role during this pathogenesis we were interested in their localization and to see if photoreceptors could be transplanted into the aged retina of MCP-1 mice.

Methods: Rod precursors were isolated from Nrl.GFP mice at postnatal day 4-5. 1 µl of the cell suspension was transplanted into the subretinal space in mice from four groups (2 month: wt (n=10); MCP-1 (n=10); 18-20 month: wt (n=5); MCP-1 (n=11)). After three weeks the eyes were processed for sectioning. The number of GFP positive, integrated cells per retina was determined. Autofluorescence Scanning Laser Ophthalmoscopy (AF-SLO), immunohistochemistry and histology were used to further characterize the animals.

Results: Between 100 and 1350 integrated rod photoreceptor cells were found in young and aged wildtype retinas showing no significant difference between these two groups. In young and aged MCP-1 mice similar numbers of integrated cells were usually seen despite a higher variability in older animals. No significant change was observed between age-matched controls and MCP-1 mice. With age, we observed the accumulation of autofluorescent spots in AF-SLO, which was more pronounced in MCP-1 than wildtype mice. By immunohistochemistry, $CD68^+$ macrophages containing autofluorescent material were identified in the interphotoreceptor matrix.

Conclusion: These results show that the age of the recipient retina does not have a detrimental effect on rod photoreceptor transplantation and that the accumulation of macrophages in the interphotoreceptor matrix of *Ccl2*/MCP-1 knockout mice does not prevent rod photoreceptor integration. These observations are encouraging for the development of photoreceptor transplantation strategies for the treatment of AMD.

EXPRESSION, PURIFICATION AND CHARACTERIZATION OF RECOMBINANT HUMAN NORRIN TOWARDS THE UNDERSTANDING OF ITS ROLE IN ANGIOGENESIS.

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Purpose: Norrie disease (ND) is a rare X-linked recessive disorder. Mutations in the NDP gene give rise to the classic ND phenotype but also exudative vitreoretinopathy (EVR), retinopathy of prematurity (ROP) as well as Coats disease. All of them display characteristic vascular defects in the retina and some also in the vitreous.
We have established cell lines in order to stably express human Norrin and disease-associated mutant variants. HEK293T cells were transfected with the respective expression constructs (wild type Norrin and four different mutant variants: p.Cys95Arg, p.Cys96Trp, p.Arg121Leu, and p.Arg121Trp) and used to express human Norrin.

Methods: Wild type and four mutant variants of Norrin were expressed in HEK293T cells. The recombinant proteins are C-terminally tagged with myc and polyhistidine for detection and purification, respectively. Proteins were extracted from the cell pellet, culture medium and extracellular matrix, separated by SDS-PAGE and immuno-detected on Western blots with an anti-myc antibody. Purification of the recombinant proteins was achieved with the His-tag and immobilized metal affinity chromatography (IMAC). The functional characterization of two Norrin variants (wild type and p.Cys95Arg) was performed by using the chicken CAM (chorioallantoic membrane) assay.

Results: All five Norrin variants were expressed and secreted in the respective HEK293T cells. The majority of Norrin was associated with the extracellular matrix (ECM) and could be released by heparin in the cell culture media. Norrin purification with IMAC was established and the identity of Norrin was confirmed by mass spectrometry. Dimerization was observed for wild type Norrin but not for the p.Cys95Arg mutant variant. In addition, preliminary results indicated an effect of Norrin on angiogenic sprouting.

Conclusions: : Our results provide information about the localization and function of Norrin and might explain the functional consequence of the p.Cys95Arg mutation. Furthermore, HEK 293T cell lines were established as a continuous source of wild type and mutant Norrin. This will provide a basis for detailed functional studies. The cell culture approach presented in this work might also be suitable for further analyses of *NDP* mutation effects. This will help to understand the molecular basis of Norrie disease and allelic disorders.

THE INFLUENCE OF BESTROPHIN ON ATP INDUCED CALCIUM-STORE DEPLETION IN RPE CELLS

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Purpose: Bestrophin, the product of the *VMD2* gene is known to function as a chloride-channel and a modulator of L-type Ca^{2+}-Channels. It is expressed in the retinal pigment epithelium (RPE) where mutations in the *VMD2* gene cause Best`s disease, an early onset macula dystrophy. Aim of this study was to investigate the influence of bestrophin on the calcium homoeostasis in RPE cells of *Vmd2*-deficient and wild-type *(wt)* mice.

Methods: RPE cells were isolated from murine eyes and held in primary culture for four weeks prior measurement. Intracellular free calcium concentration ($[Ca^{2+}]_i$) was monitored using the Fura-2 Ca^{2+} imaging method.

Results: RPE primary culture and ARPE-19 cell line showed a similar pattern of ATP (100nM) induced $[Ca^{2+}]_i$ responses. RPE cells from Vmd2 deficient mice showed a faster Ca^{2+}-response compared to RPE cells from *wt* mice. Furthermore emptying thapsigargin sensitive intracellular Ca^{2+} stores (endoplasmic reticulum) reduced the ATP evoked increase in $[Ca^{2+}]_i$ more efficiently in RPE cells of wt mice than in RPE cells of *Vmd2*-deficient mice whereas the depletion of bafilomycin sensitive Ca^{2+}-stores (acidic organelles) reduced the ATP evoked increase in $[Ca^{2+}]$i more efficiently in RPE cells of *Vmd2*-deficient mice.

Conclusions: These results seem to support our theory, that Bestrophin is involved in linking the Ca^{2+} storage to the endoplasmic reticulum.

ACTIVITIES OF CALPAIN AND PARP DURING *RD1* MOUSE RETINAL DEGENERATION

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Purpose: Retinitis Pigmentosa (RP) is an inherited blinding disease that is characterized by a progressive loss of photoreceptors. The underlying degeneration mechanisms are poorly understood and so far there is no treatment available. Excessive activation of calpain type proteases and poly-ADP-ribose-polymerase (PARP) has been shown to be involved in a number of neurodegenerative disorders. Previously we have identified an increased activity of these enzymes during photoreceptor cell death in the *rd1* mouse model for RP.

Methods: To investigate the possibility of a causal connection between excessive activity and cell death, we studied activities of calpain and PARP and the effects of specific inhibitors on short-term and long-term photoreceptor viability *in vitro*, on *rd1* retinal explants, and *in vivo* using intravitreal injections.

Results: Activation of calpain correlates and colocalized with PARP activation in *rd1* photoreceptors. The use of highly specific inhibitors for either calpains or PARP was beneficial for photoreceptor viability. PARP inhibition did not affect activation of calpains but, interestingly, calpain inhibition reduced PARP activation, suggesting that calpains somehow mediated PARP activation. PARP activity in turn co-localized with markers for oxidative stress and nuclear translocation of apoptosis inducing factor (AIF).

Conclusions: Taken together, the data suggest that calpain may mediate cell death via the activation of PARP, a subsequent energetic collapse and AIF nuclear translocation. The neuroprotective effects observed in the *rd1* mouse model for retinal degeneration suggest a therapeutic potential for certain calpain inhibitors for the treatment of RP.

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EPIDEMIOLOGICAL STUDY OF INHERITED RETINAL DEGENERATIONS IN TÜBINGEN UNIVERSITY EYE CLINIC – CURRENT RESULTS

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PURPOSE: To assess characteristics of patients with inherited retinal degenerations (IHD) seen in the Tübingen University Eye Clinic, Germany.

METHODS: Records of patients with IHD, treated at Tübingen Eye Hospital during 1994-1999, were obtained from database and reviewed. Patients with macular dystrophy (MD), Stargardt (ST) disease, Bardet-Biedl Syndrome, Usher syndrome (USH) I and II types, Central Areolar Choroid dystrophy, Choroideremia were included. Age, sex, age of diagnosis, age of visual acuity (VA) decrease, ages of night blindness and photophobia onset, types of visual field (VF) defects and age of its onset, color problems, best corrected VA data, main diagnosis were analyzed.

RESULTS:A total 259 patients were observed. Men/women ratio was 2:1. Mean age of the patients was 47,24 (SD=15,63) years old. The age distribution showed a maximum peak in 0-40 years old (40%), 36% in the 41-60 age group, 24% - above 60 years old. 29% patients were first diagnosed being in the age of 21-30, when 29% of them had VF defects and 24% - VA decrease; 9% - were diagnosed before 10 years old. Peak of night blindness and photophobia onset for 32% and 25% of patients respectively were shifted to age of 11-20. 74% of the patients had concentric constriction of VF, 21% - had central scotoma, 5% - ring scotomas. 54% of the subjects had color vision problems. Half of patients preserved best corrected VA in a better eye at the level of ∃0.5, 31% - had 0.5>VA<0.1, 12% - had VA #0.1; 7% were diagnosed as legally blind, which is corresponding to VA<0.05 according WHO definition (1992). The most frequently diagnoses were ST (34%), USH II (22%), MD (18%), Choroideremia (14%), USH I (7%).

CONCLUSION: Night blindness and photophobia were the earliest symptoms of IHD. Patients remained undiagnosed until more evident symptoms such as VA decrease and VF defects appeared. Most common VF defects were concentric constriction and central scotoma. Half of the patients preserved VA at the level ∃0.5 and 7% of patient's population were legally blind. STD, USH type II, MD and Choroideremia were most frequent diagnosed.

THE ROLE OF TWO SINGLE NUCLEOTIDE POLYMORPHISMS (SNPS) IN THE EXTENDED VMD2 PROMOTOR REGION TOWARDS HETEROGENIC PHENOTYPES IN BEST VITELLIFORM MACULAR DYSTROPHY

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Purpose: VMD2 mutations are associated with Best vitelliform macular dystrophy (BMD), an early-onset autosomal dominant retinal disorder. Manifestation and penetrance in BMD is highly variable, exhibiting intra-familial heterogeneity of clinical phenotypes even in carriers harbouring an identical pathogenic mutation. The aim of this study is to evaluate the role of two SNPs mapping to a novel regulatory sequence region of the VMD2 promotor, towards their role in variable expressivity in disease manifestation.

Methods: In silico analysis of the human VMD2 promotor was performed on the Genomatix software platform. In addition, the ECR Browser algorithms were applied to extract additive genomic regulatory elements (RE) based on the comparisons of multiple vertebrate genomes. The novel putative REs were analysed by luciferase reporter assays towards their properties to regulate gene expression. To investigate the luciferase assay derived data in vivo, transgenic Xenopus laevis tadepoles were generated, carrying the GFP reporter gene under the control of various VMD2 promotor sequence variations. In the next step SNPs were extracted from public databases and were aligned to the human VMD2 promotor sequence. Finally the influence of these SNPs on transcription factor TF binding sites was investigated by luciferase reporter assay and electrophoretic mobility shift assay (EMSA).

Results: Bioinformatic analysis revealed five regions with putative regulatory functions in the upstream region of the human VMD2 transcription start site. Strong promotor activity was observed in one of these regions, presenting an additional promotor element beside the known minimal promotor. In transgenic Xenopus laevis tadepoles eye specific GFP expression was observed under the control of the known minimal and novel VMD2 promotor elements. Two highly frequent SNPs mapping to this new RE, lead to alterated promotor activity in luciferase reporter assay and showed variable TF binding properties in EMSAs as well.

Conclusions: We have identified five conserved REs in the human VMD2 promotor region. One of these novel elements revealed high promoter activity in vitro and in vivo. Interestingly two highly frequent SNPs within this sequence did affect the promotor activity and may play a crucial role in the variable disease manifestation.

IMPAIRED MEMBRANE TARGETING AS A MAJOR EFFECT OF MUTATIONS IN *CNGA3* RESULTING IN ACHROMATOPSIA

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Purpose: Achromatopsia is an autosomal recessively inherited disease, which can be caused by mutations in *CNGA3*, a gene encoding the A3 subunit of cone cyclic-nucleotide gated channels. Patients present with a reduced visual acuity, photophobia, strongly impaired colour vision and nystagmus. Symptoms are less severe in the incomplete type of achromatopsia as compared to the complete type. Cone CNG channels are heterotetramers consisting of two A3 and two B3 subunits, which are gated by the cGMP levels in cone outer segments and enable a sodium and calcium influx into the photoreceptor cell.
In our study, we analysed the effect of four CNGA3 mutations (E228K, R283Q, R439W and R563C) on the calcium permeability, the ligand-sensitivity and protein trafficking/folding of mutant channels and compared these findings with clinical disease expression

Methods: The mutant channels were expressed in HEK293 cells for functional and immunocytological studies. Calcium imaging measurements were done with the calcium sensitive fluorescent dye fura-2 and the membrane permeable agonist 8-Br-cGMP. Patch clamp analyses were conducted in the inside-out configuration. The potassium currents recorded after application of different cGMP concentrations were used to establish dose-response relationships. Additionally, we performed co-localisation experiments to assess the integration of mutant channels into the plasma membrane.

Results: All four channel mutants showed to be functional in calcium imaging measurements although calcium influx mediated by mutants R283Q and R439W was detectable only after incubation of the HEK293 cells at 27°C instead of 37°C.
Patch clamp analyses revealed that the apparent ligand sensitivity of heteromeric mutant channels were similar to the wild type and thus not affected by the mutations E228K, R283Q, R439W and R563C. By immunocytochemical analyses we could show that only a small fraction of labelled channels co-localised with the plasma membrane after incubation of the cells at 37°C. This distribution was changed significantly after the incubation of the cells at 27°C, where we could observe a strong co-localisation of mutant channels with the cell membrane. Accordingly, we propose impaired protein folding and/or trafficking as the main pathogenic effect of the four studied mutations.
With respect to clinical findings, patients carrying the mutations R283Q and R439W were mostly diagnosed with complete achromatopsia. By contrast, patients with the mutations E228K and R563C mostly present with incomplete achromatopsia.

CONE DEGENERATION IN R91W MOUSE

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Purpose: To analyze cone cell death in a recently developed mouse model for human blinding disease caused by the R91W mutation in Rpe65 (Samardzija et al., 2008).

Methods: The mouse strains used were: Rpe65$^{-/-}$, R91W, and wild-type. For in vivo monitoring of cone degeneration the three strains were intercrossed with a strain expressing GFP in cones (coGFP) to produce Rpe65$^{-/-}$;coGFP and R91W;coGFP double mutants.
We analyzed: 1) morphology by microscopy, 2) cone survival by RT-PCR and immunofluorescence, 3) cone function by electroretinography (ERG), 4) cone degeneration in vivo in coGFP double mutants by scanning laser ophthalmoscope (SLO).

Results: Transcriptional levels of cone markers were reduced already at 4 weeks of age in R91W mice as compared to wild-type. Based on protein expression the most prominent cone loss occurred between 4 and 16 wks of age preceding the peak of rod cell loss. Cone cell degeneration was accompanied by adequate loss of function. By SLO analysis of animals with cones labeled with coGFP we were able to monitor *in vivo* the spacio-temporal cone loss and compare the effects of the two mutations (absence vs. missense) on the cone survival.

Conclusion: The gradual and selective cone loss accompanied by equivalent functional decline makes R91W pertinent to analyze the mechanisms of cone cell degeneration.

CALPAIN INHIBITORS AS A TREATMENT FOR RETINAL NEURODEGENERATION IN AN RP MODEL, THE RD1 MOUSE

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Purpose: The rd1 mouse is a well established animal model for the study of human inherited retinal degenerations such as Retinitis Pigmentosa. This model has a mutation in the , subunit of the rod cGMP phosphodiesterase (β-PDE) gene, which results in chronically opened cGMP-gated cation channels and a consequent overload of the rod cells with Ca^{2+}. Activation of the calcium-dependent protease calpain has been suggested to play a role in the cell death in this animal model.
Here, we studied the effects of two different calpain inhibitors, calpain inhibitor XI (CI XI) and calpastatin peptide (CS), on in vitro rd1 retinal cultures.

Methods: Retinae from rd1 and wt animals were cultured via an organotypic explant system under various treatment paradigms testing for the effects of calpain inhibitors in the different retinal layers. Cultures were started at post natal day (p) 5, and the inhibitors were added to the medium two days after. At p11 the cultures were stopped and processed for further experiments. TUNEL assay was used to stain for dying cells in the retina and this was combined with an in situ calpain activity assay.

Results: The application of CS to cultured rd1 retinae showed a decrease in TUNEL positive cells in the ONL, comparing with non-treated ones. On the other hand, high concentrations of CI XI induced an increase of cells dying in the photoreceptor layer. No adverse reaction was observed in wild type retinae using CS, but CI XI treatment resulted in a marked increase of TUNEL positive cells. A similar picture was obtained with calpain activity assay: activated calpains were decreased in the case of the CS treatment but increased in cultures treated with CI XI.

Conclusion: Our results imply that calpains are related with the cell death pathway in the rd1 retina. The fact that CS treatment is beneficial while CPI XI treatment is detrimental could be due to calpain isoform specific effects. Previous experiments had shown a prevention of the disease when the CI XI was used in an acute application paradigm. Therefore the relation dose-treatment period could be important.

MURINE MÜLLER-CELLS ARE POSTNATAL RETINAL PROGENITOR CELLS

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Background: Radial glia cells constitute the majority of progenitors during neurogenesis. Recent studies demonstrated that isolated rat Müller-cells - the principal glia cell population of the retina - display cardinal features of retinal progenitor cells, i.e., they self-renew and generate all neuronal cell types of the retina. Although this is true for rat Müller-cells there is no evidence for murine Müller-cells as retinal progenitor cells. Our study characterized murine Müller-cells as postnatal retinal progenitor cells for the first time.

Methods: Müller-cells were isolated from mouse retina as described previously. Isolated Müller-cells were cultivated in serum-containing medium and were investigated for the expression of Müller-cell markers by RT-PCRs and immunocytochemistry. For selection of murine Müller-cells, isolated cells were cultivated in glucose-free cell culture medium supplemented with sorbitol. In mice only glia cells can metabolize sorbitol as main carbohydrate source. For dedifferentiation into retinal progenitor cells, murine Müller-cells were cultivated in serum-replacement medium supplemented with FGF-2 and EGF.

Results: *Isolated Müller-cells expressed Müller-cell markers like GFAP, vimentin and glutamine synthetase (GS) and could be proliferated in serum-containing medium for more than 8 passages. In contrast to murine bone marrow cells, isolated Müller-cells could be maintained in glucose-free cell culture medium supplemented with sorbitol. During early growth phase, murine Müller-cells produced connective tissue like membranes instead of neurospheres after dedifferentiation in serum-replacement medium. Here, gene expression of connective tissue markers was increased. However, during late growth phase, Müller-cells formed neurospheres, a typical feature of retinal progenitor cells. These neurospheres differentiated into neuron-like cells after cultivation on laminin/ornithin cell culture substrate.*

Conclusions: This study demonstrated that murine Müller-cells can dedifferentiate into retinal progenitor cells. Murine Müller-cells are therefore interesting candidates for stem cell based therapy approaches in mouse models of retinal degeneration.

PLASMALEMMA VESICLE ASSOCIATED PROTEIN (PLVAP), A MAJOR COMPONENT OF VASCULAR FENESTRAE, IS OVEREXPRESSED IN NORRIN DEFICIENT MICE.

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Purpose: To investigate the molecular pathophysiology caused by a mutant Norrin *(Ndph)* gene, which is associated with various forms of blindness (vasoproliferative vitreoretinopathies) in humans. Mouse transcriptome analyses revealed several molecules which might be involved in early pathogenic events. One of them, Plvap (plasmalemma vesicle-associated protein), a key molecule of vascular fenestrae, was examined on protein and transcript levels during early postnatal retinal development in mutant versus wild type mice.

Methods: Quantitative RT-PCR for *Plvap* and additional transcripts was performed on retinal RNA from different developmental stages (postnatal days 5, 7, 10, 15, 21). Eye cryosections of various developmental stages (P3 onwards) of $Ndph^{y/-}$ and wild type mice were co-immunolabeled with antibodies directed against Plvap and Collagen IV.

Results: While Plvap consistently localizes to the choroid vessel system in both wild type and knockout mice at all developmental stages investigated, expression in the retinal vasculature has only been detected in $Ndph^{y/-}$ knockout but not in wild type mice. This Plvap overexpression was already observed at postnatal day 3 (p3), a point in time when blood vessel development is initiated in the mouse retina.

Conclusions: Early ectopic expression of Plvap in Norrin knockout mice may explain the leakiness of retinal blood vessels in mutant mice and result in impaired angiogenesis and hypoxia. A further up-regulation of Plvap in later development could not only be caused by hypoxia-induced overexpression of VEGFA, but also by a dysfunctional vasculature itself.

COMBINED MRNA AND MIRNA EXPRESSION PROFILING OF THE *CPFL1* MUTANT – A MOUSE MODEL OF CONE DYSTROPHIES

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The *cpfl1* mutant *(cone photoreceptor function loss 1)* is a mouse model carrying mutations in the cone specific phosphodiesterase 6C *(pde6c)*. 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.
Expression analysis of *cpfl1* 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 $\leq$0.05) were obtained and gene regulation networks were generated by the Ingenuity Pathways Analysis software. To verify the data 11 transcripts were analyzed by qRT-PCR using the LC 480 system.
338 transcripts were differentially regulated in the retinas of 4 week old mice and 223 in those of 8 week old mice. There was an overlap of 30 % between both experiments. A large number of genes encoding proteins involved in phototransduction were down regulated. Gene regulation networks revealed misregulation of genes associated with cell death, proliferation and gene expression. All the transcripts chosen for Real-time validation could be verified. In the miRNA array analysis of 4 week old mice we found differently regulated miRNAs which have potential target genes included in the differential gene list of our previous transcriptional analysis. Among these is miR182 which has been shown to be expressed extensively in retinal tissue and has 8 potential target genes including immune responsive elements.
The expression analysis of the *cpfl1* mutant highlighted a clear misregulation of phototransduction 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 degeneration.

SPLICING OF *RPGR* AS A MODIFIER OF RETINITIS PIGMENTOSA.

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Purpose: Retinitis pigmentosa (RP) is a hereditary disorder which causes degeneration of photoreceptors and often leads to blindness. *RPGR* is mutated in approximately 70% of all X-linked RP cases. Interestingly, splicing of *RPGR* produces several alternative transcript variants. We aim to understand the influence of *RPGR* splicing on the pathogenesis of RP and other RPGR-associated disease phenotypes.

Methods: Lymphoblastoid cell lines derived from RP patients with *RPGR* mutations were screened by RT-PCR. Mutation-induced changes in expression levels of *RPGR* transcripts were quantified by real-time RT-PCR, and their amount was determined in multiple tissues from healthy individuals (retina, brain, lung, liver, testis)

Results: We analyzed splicing of *RPGR* in sixteen cell lines from patients with different *RPGR* mutations. In one of these cell lines, a four-fold increase in expression of a novel exon termed 11a was detected. In this exon, we identified a novel sequence alteration which is predicted to generate an additional exonic splicing enhancer binding site and thus may explain the increased expression levels. Interestingly, multi-tissue expression analysis showed highest levels in retina. In addition to these splice alterations, a missense mutation affects exon 5.
In a second cell line carrying a 4 bp deletion in exon 11, we also identified an *RPGR* splice defect. Increased levels of a novel transcript isoform which skips exon 12 were detected. This splice defect abolishes the frameshift caused by the 4 bp deletion. In contrast, transcripts that include exon 12 are subject to nonsense-mediated decay, and thus are degraded. Furthermore, highest levels of exon 12 skipping were found in lung compared to other tissues, which may indicate a tissue-specific effect.

Conclusions: TOur results support that splice defects in *RPGR* influence the pathogenesis of RP and may act as modifiers of the disease phenotype, not only in the retina.

STRUCTURAL AND FUNCTIONAL CHANGES IN PATIENTS WITH TYPE 2 MACULAR TELANGIECTASIA OVER TIME

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Purpose: In a cross-sectional analysis of patients with type 2 macular telangiectasia (MacTel), we have previously demonstrated severe localized dysfunction next to fixation despite good central visual acuity and that scotopic loss was deeper and larger compared to photopic scotoma (ARVO 2007). Here, we investigate structural and functional changes over a one year review analysis.

Methods: As part of the MacTel-Study Project, a cohort of patients (median age 66.5 years, range 44-74) with MacTel underwent repeated scotopic and photopic fine matrix mapping (FMM), 10-2 photopic microperimetry (Nidek MP-1, MP) and imaging studies including fundus photography, fluorescein angiography, optical coherence tomography and scanning laser ophthalmoscopy imaging (exc. λ= 488 nm, em.>500 nm and exc. λ= 790 nm, em.>810 nm). Retinal sensitivity was compared with individual baseline results and the number of test points with loss ? 10 dB with FMM compared to age-matched control values was determined.

Results: ETDRS visual acuity assessment showed a mean difference between examinations of 0.67 letters (range -5 to 8). The mean difference of sensitivity values of all test points was 3.3 dB (0.4 to 5.5) for microperimetry, 0.5 dB (-0.8 to 1.7) for photopic and -2.2 dB (-6 to 1) for scotopic FMM, respectively. The difference in number of test points with loss of $\geq$ 10 dB was significantly higher for scotopic than for photopic testing ($p = 0.04$, Wilcoxon Signed Ranks test). Small progression of scotoma correlated with slight increase in retinal blood vessel dilatation and hyperfluorescence and subtle enlargement of pigmented plaques.

Conclusions: In this cohort, changes in central visual acuity and MP testing after one year most likely do not extend beyond test-retest variability. The deterioration of scotopic sensitivity confirms our previous results of more severe rod compared to cone dysfunction in MacTel. Changes in function testing over a one year period may be useful parameters in assessing disease progression and in monitoring intervention studies in patient with MacTel.

THERAPEUTIC STRATEGY TO RESCUE MUTATION-INDUCED EXON SKIPPING IN RHODOPSIN BY ADAPTATION OF U1 SNRNA.

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Purpose: Splice site recognition is a key step in the splice process. Hence, splice site mutations constitute a major cause of disease-associated mis-splicing in numerous diseases. Here, we tested a U1 snRNA-mediated therapeutic strategy to treat mis-splicing caused by a novel mutation in rhodopsin (RHO).

Methods: Mutational screening was performed by direct sequencing of genomic DNA from Retinitis pigmentosa (RP) patients. Site-directed mutagenesis was applied to introduce sequence alterations in minigenes and U1 snRNA. Splice assays were performed by cotransfection of minigenes and U1 snRNA expression constructs in cell culture (COS 7) and mouse retinal explants. Quantitative RT-PCR was applied to determine the efficiency of the therapeutic strategy.

Results: We identified a novel donor splice site mutation at the last nucleotide of exon 4 of RHO (c.936G>A). The mutation is a silent exchange that causes mis-splicing of RHO in cultured cells and retinal explants. Skipping of exon 4 and activation of a cryptic splice site produced two different mis-spliced transcripts. Both transcripts are predicted to result in truncated proteins explaining the RP phenotype diagnosed in the patient.
Donor splice sites are recognized by complementary base pairing of U1 snRNAs to pre-mRNA. We tested the feasibility of adapted U1 snRNA as therapeutic strategy to rescue the splice defects in RHO. Quantitative transcript analysis showed that exon 4 skipping was decreased to wild type levels by mutation-adapted U1 snRNA. Nevertheless, activation of the cryptic splice site was still detected. Cryptic splice site inhibition restored accurate splice site recognition and revealed 89% rescue of exon skipping. Highest efficiencies were observed with U1 snRNA adapted to the constitutive splice site sequence.

Conclusion: Our results demonstrate for the first time the feasibility and high efficiency of U1 snRNA-mediated therapeutic interventions to treat donor splice site mutations that affect the last nucleotide of an exon. These findings have implications on various diseases caused by similar mutations.

PHOTORECEPTOR CELL SPECIFIC KNOCK OUT OF HYPOXIA-INDUCIBLE-FACTOR 1α IN A MODEL OF HYPOXIA DEPENDENT RETINAL NEUROPROTECTION

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Purpose: Hypoxic preconditioning is an established method to protect cells from various degenerative stimuli. In our model of induced retinal degeneration it protects completely from photoreceptor cell death after light exposure. During preconditioning hypoxia-inducible-factor 1α (HIF-1α), the most important transcription factor responding to hypoxia is stabilized in the retina and differentially regulates the expression of a large number of target genes. Here we analyze whether photoreceptor-specific expression of HIF-1α is required for neuroprotection.

Methods: A systemic knock out of HIF-1· has been proven to be lethal. Therefore, we used floxed HIF-1α mice which were intercrossed with tamoxifen (TAM) inducible photoreceptor-specific cre mice (Prp-Cre). TAM (or Oil as control) was administrated twice a day, 5 days in a row (1mg/40g mouse) in adult mice to induce the knock out. Knock out efficiency was determined by analyzing gene expression using real time PCR and western blot. Hypoxic preconditioning was performed for 6 h at 6% O_2. Hypoxic preconditioned or normoxic mice were exposed to 13'000 lux for 2 h. Cell death was quantified by the measurement of free nucleosomes and by morphology.

Results: The knock out of HIF-1α in photoreceptors reduced HIF-1α mRNA by about 70 %. Immunoblotting showed a significant reduction of HIF-1α protein levels in hypoxic preconditioned mice and HIF1-α target genes were less induced than in control mice. These data suggest that HIF-1α was successfully knocked-out in photoreceptors. The analysis of retinal cell death in light exposed knock out mice after hypoxic preconditioning indicated that HIF-1α ablation in photoreceptor cells had no significant effect on retinal neuroprotection.

Conclusion: Prp-Cre mice are a powerful tool to eliminate gene expression in photoreceptor cells. Here, we successfully deleted HIF-1α from photoreceptor cells, which led to a significant reduction of HIF-1α dependent expression of target genes. However, retinal neuroprotection was not affected suggesting that protection of photoreceptors by hypoxic preconditioning does not depend on HIF-1α stabilization in these cells. It might be possible that stabilization of HIF-1α in cells of the INL and/or GCL contributes to neuroprotection. Additionally, the induction of other HIF family members like HIF-2· could potentially compensate for the lack of HIF-1α.

LEBER CONGENITAL AMAUROSIS: CLINICAL CHARACTERISTICS OF GENOTYPED PATIENTS AND FULL-FIELD ERG ABNORMALITIES IN PARENTS OF AIPL1, CRB1 AND CEP290-RELATED SUBJECTS

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Aim: To describe the clinical characteristics of 4 genotyped Hungarian patients with Leber Congenital Amaurosis and compare with phenotypes described in the literature. To study clinical and electrophysiological findings in the patients' parents.

Patients: 3 children (aged 4 to 6 ½ years) and one adult (aged 29 years) with congenital blindness or severe visual impairment underwent clinical, electrophysiological and genetic examination. In the parents (6 asymptomatic adults ranging in ages from 32 to 48 years) clinical and electrophysiological examinations were performed.

Methods: Ophthalmological examination included testing of visual acuity, fundoscopy and digital fundus photography. In the adult patient optical coherence tomography was also performed. Full-field electroretinography was carried out on all participants. Molecular genetic analysis included screening for 10 known genes involved in LCA.

Results: Patient 1, 2 and 4 have a visual acuity of light perception (LP)-counting fingers (CF)-hand motion (HM), which remained unchanged during follow up. At the first examination, four years ago Patient 3 had a visual function of 0.1 / 0.16 which showed slow deterioration. There was a remarkable maculopathy in Patients 1, 2 and 3. Nummular pigmentation was in Patient 3 and 4 and diffuse white dots in Patient 3 and mid-peripheral confluent white flecks in Patient 4.
Full-field ERGs were undetectable in all patients. Genetic screening revealed AIPL1 mutation in Patients 1 and 2, CRB1 mutation in Patient 3 and CEP290 mutation in Patient 4.
Parents are asymptomatic and 5 have no remarkable fundus findings. There are widespread alterations in the electroretinograms; cone-driven ERGs are more effected than rod-driven responses. Carriers of AIPL1 and CRB1 mutations have subnormal and parents of heterozygous patients with CEP290 mutation have supernormal b-waves.

Conclusions: Our findings confirm the previously described phenotypes in all 3 gene mutations. Supernormal full-field ERGs in parents of CEP290-related patient remains a question for further evaluation.

LOW RETINAL DOCOSAHEXAENOIC ACID (DHA) LEVELS ARE LINKED TO MICROGLIA ACTIVATION IN RETINAL DEGENERATION

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Purpose: We have previously identified strong microglia activation preceeding photoreceptor apoptosis and retinal degeneration in the mouse model for human X-linked juvenile retinoschisis (Rs1h$^{-/Y}$) (Gehrig et al., 2007; Weigelt et al., 2007). Our aim was to further characterize microglia activation in retinoschisin-deficient as well as rhodopsin-knockout mice with an emphasis on inflammatory lipids and the retinal phospholipid composition.

Methods: *In situ* microglia activation in the retina was studied using transgenic EGFP-expressing MacGreen mice and fluorescent dyes to stain lipid bodies. The influence of DHA on activated microglia gene expression was tested in vitro. Total and phospholipid fatty acid composition of retinae from RS1h$^{-/Y}$, Rho$^{-/-}$, and WT mice were measured by electrospray ionization tandem mass spectrometry (ESI-MS/MS). A pilot experiment was performed for DHA supplementation in RS1h-/Y retinae.

Results: A strong expansion of amoeboid microglia containing arachidonic acid rich lipid bodies was detected in the P14 retina of MacGreen x RS1h$^{-/Y}$ mice. Evaluation of the fatty acid composition in RS1h$^{-/Y}$ and Rho$^{-/-}$ mice retinae revealed significant lower levels of the ω3-polyunsaturated fatty acid docosahexaenoic acid (DHA) at P14 and P18, respectively. Comprehensive gene expression profiling identified that the reduced DHA levels coincide with overexpression of microglia activation markers. Furthermore, exogenous DHA was able to block LPS-induced activation of BV-2 microglia cells *in vitro*. Based on these data, we designed an animal experiment for dietary supplementation of DHA to study the potential blocking effects on microglia activation and retinal degeneration. As a first result of the ongoing study, we could show a significant systemic and retinal enrichment of the ω-3 fatty acid DHA in RS1h$^{-/-}$ mice.

Conclusions and Perspectives: Our work indicates that low DHA levels in the retina of young RS1h$^{-/Y}$ and Rho$^{-/-}$ mice coincide with increased microglia activity. A dietary supplementation study is under way to analyze the potential benefical effects of DHA in attenuating microglia activation and retinal degeneration.

References:
Gehrig A, Langmann T, Horling F, Janssen A, Bonin M, Walter M, Poths S, Weber BH. 2007.

Genome-wide expression profiling of the retinoschisin-deficient retina in early postnatal mouse development. Invest Ophthalmol Vis Sci 48:891-900.
Weigelt K, Ernst W, Walczak Y, Ebert S, Loenhardt T, Klug M, Rehli M, Weber BH, Langmann T. 2007. Dap12 expression in activated microglia from retinoschisin-deficient retina and its PU.1-dependent promoter regulation. J Leukoc Biol 82:1564-74.

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A NOVEL CONSTITUTIVELY ACTIVE RHODOPSIN MUTATION CAUSES AUTOSOMAL DOMINANT CSNB

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Purpose: Mutations in *RHO, PDE6B and GNAT1* can lead to autosomal dominant congenital stationary night blindness (CSNB). Constitutive activation of the phototransduction cascade has been suggested to be the pathogenic mechanism underlying this disease. Here we aimed to identify the genetic defect in a large Swiss family affected with autosomal dominant CSNB and to investigate the pathogenic mechanism of the disease-causing mutation.

Methods: Two affected cousins of a large Swiss family were clinically examined. In addition to standard ophthalmological examinations, funduscopy, EOG, ERG and dark adaptometry were performed. For genetic diagnosis, the coding exons and flanking regions of *RHO* were PCR amplified and sequenced in the DNA of 7 affected and 5 unaffected members of this family. The ability of mutant rhodopsin to constitutively activate transducin was monitored by measuring the catalytic exchange of bound GDP for radiolabeled [^{35}S]GTPγS in transducin.

Results: We identified a novel mutation in *RHO* (c.884C>T, p.Ala295Val) in patients with adCSNB. Two affected cousins were examined clinically. They had full vision under photopic conditions, showed no fundus abnormalities, revealed EOG results in the normal range but presented night blindness. Only the scotopic ERG of both patients was altered. One patient revealed an electronegative ERG due to a severely reduced b-wave, while the affected cousin had no electronegative ERG but also a reduced b-wave. In both, the oscillatory potentials were severely reduced. *In vitro* studies in the presence of 11-*cis* retinal showed that the mutant rhodopsin is inactive, similar to wildtype, responding only when exposed to light. However, in the absence of 11-cis-retinal, unlike wildtype opsin, the mutant opsin constitutively activates transducin.

Conclusions: Our study adds a fourth rhodopsin mutation associated with CSNB. Although the phenotype of autosomal dominant CSNB may vary slightly in patients showing mutations

in *RHO, PDE6B or GNAT1*, the disease course seems to be stationary with only scotopic vision being affected. Our data indicate that the mutant opsin is able to constitutively activate transducin, which is a consistent and common feature of all four CSNB-associated rhodopsin mutations reported to date.

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