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RESEARCH CONFERENCES

ESF-UB Conference in Biomedicine
in association with
EuroVisionNet and Fondation Voir et Entendre

Rare Diseases II: Hearing and Sight Loss

Hotel Eden Roc, Sant Feliu de Guixols (Costa Brava) Spain
22 - 27 November 2009

Chairs:
Christine Petit, Institut Pasteur, Paris, FR &
José-Alain Sahel, Institut de la Vision, Paris, FR

Organising Committee:
Olivier Lorentz, Institut de la Vision, Paris, FR
Michèle Roa, Institut Pasteur, Paris, FR

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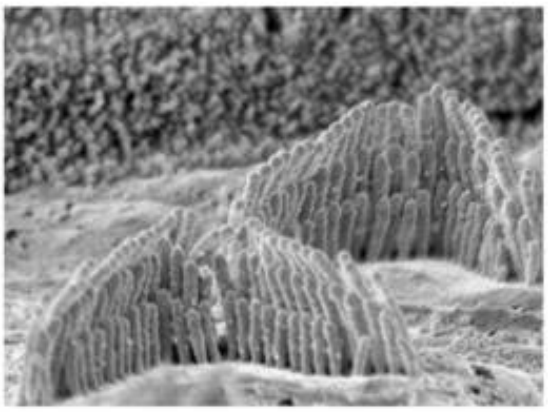

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Welcome address

Ladies and Gentlemen,
Dear Colleagues and Guests,

It is our great honour and pleasure to welcome you to the Hearing and Sight Loss Research Conference.

Following the tradition of the European Science Foundation, the Conference revolves around original research works that will present to the scientific community a broad range of new information on the relationship between the auditory and visual sensory systems. The Conference will provide an insight on the significant progress in understanding the pathogenesis of the hearing and sight loss, and the recent advances in the development of therapeutic approaches.

Keynotes lectures reflecting the ongoing efforts towards understanding, prevention and cure of the hearing and sight loss will be held by 45 invited speakers, opinion leaders in the field of rare diseases affecting the vision and hearing.

The scientific program also includes eight selected short talks and 51 posters covering both basic and clinical science. They will be presented by 86 participating scientists from 22 countries. We are happy to have a lot of young scientists.

Apart from a most comprehensive list of state-of-the-art lectures and scientific presentations, the Organizing Committee has also put together a wonderful meeting place and lively social program for the benefit of our distinguished speakers and delegates.

We hope that the Conference affords an excellent opportunity to exchange of ideas and opinions, to communicate scientific information, to get and improve knowledge, to initiate new collaborations and friendship.

Wishing you all a fruitful, professionally stimulating and personally rewarding Conference,

The Organizers

Scientific committee

This conference is organised by the European Science Foundation (ESF)
www.esf.org/conferences
The conference website is: www.esf.org/conferences/09295

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General information

Meeting venue
Hotel Eden Roc, Sant Feliu de Guixols (Costa Brava), Spain
Phone: +34 972 320 100
Fax: +34 972 821 705
Email: eden@caproig.com
Web: http://www.caproig.com/index.asp?hotel=ER&idioma=4

Participants' registration includes:
Admission to the Scientific program and Abstract book, Registration and meeting costs, Conference bag, Accommodation, Lunch, Dinner and Coffee breaks, Welcome ceremony, Closing ceremony, Excursion to Girona, Poster session, Group transportation from/to the nearest airport on arrival and departure days.

Posters:
Posters can be mounted from 22 November. They will be displayed throughout the meeting. Posters can be fixed with self-adhesive tape, blue-tack or drawing pins onto double-sided poster panels. Recommended poster size is 100 cm high x 70 cm wide.

Short talks:
Short talks will take a maximum of 15 min each. It is suggested to employ most of the slot (~10 min) for the presentation and leave ~5 min for discussion.

Acknowledgements

The Organizing committee wishes to thank the following companies for their support in terms of the conference organizational needs: European Science Foundation, University of Barcelona, Generalitat de Catalunya, EuroVisionNet, Fondation Voir et Entendre, and EuroHear.

Scientific programme

Sunday, 22 November	
Late afternoon / early evening	Registration at the ESF-RC desk
19.00	Welcome Drink
20.00	Supper
Monday, 23 November	
08.30-09.00	**Conference opening** Christine Petit Institut Pasteur, Paris, FR **Presentation of ESF** Isabel Varela-Nieto Universidad Autonoma de Madrid, ES ESF Rapporteur
Chairs: Adam Sillito, Institute of Ophthalmology, London, UK**, Robert V. Shannon,** House Ear Institute, Los Angeles, US	
09.00-10.20	**Process of the sensory information in the two sensory systems** Yves Frégnac Institut de Neurobiologie Alfred Fessard, Gif-sur-Yvette, FR *Reading out the neural code by listening to the synaptic echoes of the visual world in a single cell* Paul Avan University of Auvergne, Clermont-Ferrand, FR *From key stages of acoustic signal processing to the clinical impact of their disruption*
10.20-10.50	Coffee break
10.50-12.30	**Visual and auditory neurophysiology** Adam Sillito Institute of Ophthalmology, London, UK *Role of motion linked feedback in visual processing* Sophie K. Scott Institute of Cognitive Neurosciences, London, UK *Acoustic speech and voice signal neural processing*
12.30	Lunch
Chairs: Alain Chedotal, Institut de la Vision, Paris, FR**, Paul Avan,** University of Auvergne, Clermont-Ferrand, FR	
13.30-14.50	**Evolution of the two sensory systems** Don Zack Johns Hopkins Hospital, Baltimore, US *Role of transcription factors in the evolution of the visual system* Hernan Lopez-Schier Centre de Regulació Genómica, Barcelona, ES *Hair-cell function through evolution*
14.50-16.10	**Development of the sensory organs** Valeria Marigo University of Modena and Reggio Emilia, IT *Development of the vertebrate retina* Donna Fekete Purdue University, West Lafayette, US

	Development of the vertebrate inner ear
16.10-16.40	Coffee break
16.40-17.20	**Neural development and wiring** Alain Chedotal Institut de la Vision, Paris, FR *Genetic dissection of hindbrain commissures*
17.20	**Inherited genetic development disorders of the retina and the cochlea** Shomi Bhattacharya Institute of Ophtalmology, London, UK *Genetics of retinitis pigmentosa with a special focus on splicing factor genes* Karen Avraham Sackler School of Medicine, Tel Aviv, IL *MicroRNAs in the vertebrate inner ear*
20.00	Dinner
Tuesday, 24 November	
Chairs: José Cunha-Vaz, AIBILI, Coimbra, PT**, Hannie Kremer,** Radboud University Nijmegen, NL	
08.30-09.50	**Visual and auditory sensory transduction** Eberhart Zrenner University of Tübingen, DE *Signal transduction in photoreceptors and related diseases* Maryline Beurg University Bordeaux II, FR *Mechanotransduction of mammalian cochlear hair cells*
09.50-10.20	Coffee break
10.20-11.40	**Signal transduction diseases** Christina Zeitz Université Pierre et Marie Curie Paris 6, FR *New insights towards the understanding of night blindness* Christine Petit Institut Pasteur, Paris, FR *Mechanosensation in auditory sensory cells: what did we learn from deafness genes?*
11.40-12.30	**From rare to complex disease** Philip J. Luthert UCL Institute of Ophthalmology, London, UK *From rare to complex disease*
12.30	Lunch
Chairs: Hélène Dollfus, CHU de Strasbourg, FR**, Ping Chen,** Emory University, Atlanta, US	
13.30-14.30	**Role of the cilium** Philip Beales Institute of Child Health, London, UK *Sensory deficits and disease mechanisms of Bardet-Biedl syndrome*
14.30-15.50	**Cilium in pathogenesis** Hélène Dollfus CHU de Strasbourg, FR *Clinical and biological overview of the retinal degeneration in*

	ciliopathies Ping Chen Emory University, Atlanta, US *Shaping the mammalian hearing organ by the planar cell polarity pathway*
15.50-16.20	Coffee break
16.20-17.00	**Usher syndrome pathogenesis** Hannie Kremer Radboud University Nijmegen, NL *Usher interactome*
17.00-18.30	**Genotype/phenotype correlation issues in rhodopsin and connexion paradigms** Isabelle Audo Université Pierre et Marie Curie, Paris, FR *Mutation spectrum of rhodopsin mutations in French autosomal dominant rod-cone dystrophy patients and their phenotype/genotype correlation* Francisco J. del Castillo Hospital Ramón y Cajal and CIBERER, Madrid, ES *Role of connexins in sensorineural hearing impairment*
18.30	Poster sessions
21.00	Dinner
Wednesday, 25 November	
Chairs: Arne Holmgren, Karolinska Institute, Stockholm, SE, **Karen Avraham,** Tel Aviv University, IL	
08.00-08.30	Bernd Fritzsch University of Iowa, US *Molecular conservation of eye and ear development*
08.30-09.30	**Function and dysfunction of ribbon synapses** Johann H. Brandstätter University of Erlangen-Nuremberg, DE *Function and dysfunction of photoreceptor ribbon synapses* Paul Fuchs Johns Hopkins University, Baltimore, US *Synaptic physiology of cochlear hair cells*
09.30-10.30	**Oxidative stress** Arne Holmgren Karolinska Institute, Stockholm, SE *Defence against oxidative stress and redox signalling* Thierry Léveillard Institut de la Vision, Paris, FR *RdCVF signaling*
10.30-11.00	Coffee break
11.00-13.00	Short Talks: Paulo Ferreira Duke University Medical Center, Durham, US *RanBP2-mediated neuroprotection is associated with the modulation of functionally diverse but linked pathways in response to oxidative stress* Tudor Constantin Badea Johns Hopkins University Schol of Medicine, Baltimore, US *Brn3a, Brn3b, and Brn3c define distinct populations of retinal*

	ganglion cell type Baerber Rohrer Medical University of South Carolina, Charleston, US *Mitochondrial capacity as a biomarker of photoreceptor cell stress* Enrique De la Rosa Centro de Investigaciones Biológicas CSIC, Madrid, ES *Systemic AAV- mediated delivery of proinsulin delays visual loss in a retinitis pigmentosa mouse model* Aziz El Amraoui Institut Pasteur, Paris, FR *The mammalian beta Heavy spectrin, an unconventional spectrin defies convention in the auditory and photoreceptor cells* Catherine Weisz Johns Hopkins University, Baltimore, US *Synaptic activity and stimulation of type II cochlear afferents* Ben Warren University of Sussex, Brighton, UK *The dynein motor is the basis of active oscillations of mosquito antennae* Natalia Caporale University of California, Berkeley, US *LiGluR-mediated visual responses in the rd1 mouse model of retinitis pigmentosa*
13.00	Lunch
14.00	Half-day excursion to Girona
20.00	Dinner
Thursday, 26 November	
Chairs: Botond Roska, Institute Friedrich Miescher, Basel, CH, **Stefan Heller**, Stanford University, US	
09.00-09.45	**Spectrum of new therapies for retinal and cochlear diseases** José-Alain Sahel Institut de la Vision, Paris, FR *Neuroprotection in rod-cone dystrophies : from bench to bedside*
09.45-10.30	Yvan Arsenijevic Hôpital Jules-Gonin, Lausanne, CH *Gene therapy for retinal dystrophies*
10.30-11.15	Botond Roska Institute Friedrich Miescher, Basel, CH *Targeted expression of light sensitive pumps and channels restores visual function in animal models of Retinitis Pigmentosa*
11.15-11.45	Coffee break
11.45-12.45	Serge Picaud Institut de la Vision, Paris, FR *Electronic retinal implants* Robert V. Shannon House Ear Institute, Los Angeles, US *Electrical stimulation of the human cochlea, auditory brainstem and midbrain*
12.45	Lunch

Chairs: Yvan Arsenijevic, Hôpital Jules-Gonin, Lausanne, CH; **Neil Segil**, House Ear Institute, Los Angeles, US	
13.30-14.00	Neil Segil House Ear Institute, Los Angeles, US *Development and regeneration of the inner ear*
14.00-14.30	Stephan Heller Stanford University, US *In vitro generation of hair cells from embryonic and induced pluripotent stem cells*
14.30-15.00	Erik C. Böttger University of Zurich, CH *From gene to drug design: mitochondrial deafness alleles and aminoglycoside ototoxicity*
15.00-15.30	Susanne Trauzettel-Klosinksi University of Tuebingen, DE *Rehabilitation: Reading training in patients with Stargardt`s disease - a randomized and controlled study*
15.30-16.00	Coffee break
16.00-16.30	Christina Fasser, *Retina Suisse - Retina International, Zurich, CH;* Steffen Suchert, *FAUN Foundation, Nürnberg, DE* *Patients' expectations*
16.30-17.30	Forward Look Plenary Discussion
17.30	José-Alain Sahel Institut de la Vison, Paris, FR *Concluding remarks*
20.00	Get-together & Conference dinner
Friday, 27 November	
Breakfast & Departure	

Invited lectures

European Science Foundation & European Medical Research Councils

Isabel Varela-Nieto
Universidad Autonoma de Madrid, Spain
Member of the ESF-EMRC Core Group & Standing Committee

The European Science Foundation (ESF) is an independent, non-governmental organisation, the members of which are 80 national funding agencies, research-performing agencies, academies and learned societies from 30 countries. The strength of ESF lies in the influential membership and in its ability to bring together the different domains of European science in order to meet the challenges of the future. Since its establishment in 1974, ESF, which has its headquarters in Strasbourg with offices in Brussels and Ostend, has assembled a host of organisations that span all disciplines of science, to create a common platform for cross-border cooperation in Europe. ESF is dedicated to promote collaboration in scientific research, funding of research and science policy across Europe. Through its activities and instruments ESF has made major contributions to science in a global context.

The European Medical Research Councils (EMRC) is the membership organisation for all the medical research councils in Europe under the auspices of the ESF. EMRC's mission is to promote innovative medical research and its clinical application towards improving human health. EMRC offers authoritative strategic advice for policy making, research management, ethics and better health services. In its activities, EMRC serves as the voice of its Member Organisations within the European scientific community. EMRC disseminates knowledge and promotes the socio-economic value of medical research to the general public and decision makers.

Each ESF activity is designed from the perspective of the research community and tailor-made to provide an opportunity for researchers to break the barriers of international borders and to come together for the benefit of European science as a whole. More information about ESF can be found on our website http://www.esf.org, funding opportunities can be found at http://www.esf.org/activities.html and calls for proposals are published at http://www.esf.org/activities/calls-and-funding.html.

Reading out the neural code by listening to the synaptic echoes of the visual world in a single cell

Yves Frégnac
U.N.I.C., UPR CNRS 2191, Gif-sur-Yvette, France

The biological foundations of visual perception in the mammalian brain show an apparent paradox: on the one hand, the functional specificity of the visual system seems to be best explained by a serial cascade of filters from retina to cortex; on the other hand, the anatomical architecture involves not only a forward cascade but also a profusion of feedback routes from higher-order processing stations as well as recurrent connections confined within each processing relay. Such is the case of the primary visual cortex (V1): most neurons have a "tubular" view of the world, responding with spikes to visual stimuli within a "receptive field" (RF) limited to a few degrees of visual angle. This is in spite of the fact that these neurons receive 95% of their synaptic input (>95%) from neurons elsewhere in V1 or other cortical areas.
Intracellular recordings *in vivo* give direct access to the subliminal responses evoked by stimuli shown in the "silent surround" of the RF. Combination of intracellular recordings and voltage sensitive dye imaging further shows the existence of slowly propagating activation waves spread at slow speed over several millimeters. Thus, the V1 network should not be considered as an ordered mosaic of independent "tubular" analyzers, but rather as a constellation of wide field integrators, integrating simultaneously echoes arising from large regions of visual space.
I will review electrophysiological findings from my lab, which led us to consider three related possibilities: 1) the intrinsic cortical connectivity may remodel processing of the visual image through the recruitment of intra-V1 "horizontal" links and feedback projections from higher cortical areas; 2) the *effective* functional architecture of the V1 network is reconfigurable by the external context provided by the "silent" periphery; 3) the various classes of eye-movements (fixation, tremor, drift, saccade), which shape the temporal spectrum of the retinal flow, dynamically alter the RF properties.
In particular, a realistic understanding of V1 function can be gained when using full-field animation of visual scenes. A recent series of intracellular recordings using movies reproducing the effects of eye-movements on the viewing of a natural scene showed that the simulated retinal flow exerts both suppressive and facilitatory effects. The comparison in the same cell of the evoked membrane potential trajectory and the spiking pattern for stimuli of different complexities demonstrates that sensory coding is highly dependent on the statistics of the sensory input. For natural scenes, the noise in cortical dynamics diminishes, the discharge pattern becomes "sparse" and spike timing precision reaches the ms range. In simpler terms, the neural code efficiency seems optimized and the dynamical behavior of the network becomes more deterministic when submitted to natural statistics. Conversely, these observations suggest that the network would become noisy-less when input statistics imposed by brain machines interface or visual stimulators meet those previously experienced during development or behavioral learning.

This work, done in collaboration has been supported by the CNRS, by grants from the ANR (Natstats) and the ACI-NIM to YF, and by the European Commission (FET- Bio-I3: contract no. 015879 (Facets)).

From key stages of acoustic signal processing to the clinical impact of their disruption

Paul Avan
University of Auvergne, Clermont-Ferrand, France

Many critical stages of sound processing occur in the cochlea and at the synapses between inner hair cells and cochlear neurons. If they fail, in addition to suffering from decreased sensitivity the patients will experience numerous distortions and an impaired ability to understand speech. The choice of appropriate rehabilitation strategy and hearing aid settings depends on correctly identifying which mechanisms have failed. These can be split into several categories according to which sensory cells are impaired. With their unique ability to exert mechanical feedback on the cochlear partition, outer hair cells amplify sound in a frequency-specific and nonlinear manner, which produces tuning and helps increase the dynamic range of encoded sounds and their signal to noise ratio, for instance by enhancing some stimuli while suppressing others. On the other hand, the synaptic apparatus of inner hair cells works in such a way that the timing of action potentials in the cochlear nerve closely respects the temporal fine structure of sound messages, up to a few kHz. If many molecules and cellular substructures involved in sound processing have been identified, scant clinical objective tools allow the faulty perception of a hearing-impaired patient to be explained and the outcome of hearing aid fitting to be usefully predicted, i.e. whether satisfactory speech intelligibility can be restored even in noisy environments. These tools include otoacoustic emissions probing outer hair cells, electrocochleography and auditory brainstem evoked potentials measuring the synchronous activity of auditory neurons in response to brief test tones. Examples will be presented of recently identified clinical patterns of sensorineural hearing loss in relation to specific genetic defects or specific patients' complaints.

Role of motion linked feedback in visual processing

Adam Sillito
UCL Institute of Ophthalmology, Bath Street, London EC1V 9EL, UK

In the central visual system feedback interactions parallel the feed forward connections and there are arguments for considering its function in terms of iterative interactions in a circuit rather than a sequence of processing steps between levels. From this viewpoint it is notable that in the primate visual system the cortical motion area MT/V5 provides a cascaded feedback influence via the primary visual cortex (V1) with the potential to influence the responses of the thalamic relay cells in the LGN. The feedback from MT to V1 exerts strong and clear effects on V1 responses to flashing and moving stimuli. A component of the feedback connections from MT terminate in the upper and lower parts of layer 6 of V1 where the cortico-geniculate neurons providing feedback to the magno and parvo cellular layers of the LGN are found. Layer 6 cells in V1 in turn influence the visual responses and response pattern of LGN cells.
All this has the surprising implication that information about stimulus motion reflected in the responses of MT cells is available to the LGN cells transferring the retinal input to V1. Moreover the receptive fields of MT cells are roughly ten times larger than those of V1 cells, they provide feedback to a retinotopic area of V1 that broadly matches the extent of the MT receptive field and this in turn, via layer 6 of V1, would exert an influence on a similar or slightly larger retinotopic area of the LGN. Thus individual LGN cells gain an “insight” into motion linked events occurring at substantial distances beyond their classical receptive field. In principle it offers a potential solution to the aperture problem at the level of the LGN.
We will discuss data indicating that the influence from MT provides a motion driven “searchlight” highlighting the path of a moving stimulus over the projected retinotopic locations in the LGN that the stimulus will engage. This is a predictive modulation of the state of LGN relay cells preparing them in anticipation of the arrival of the stimulus. It is also clearly an iterative process where the representation of the visual world sits in the fluxing interactions of the circuit. The clinical significance of this is that this iterative and predictive influence will be damaged in patients with significant retinal lesions and so the function deriving from normal areas of their retina will also be damaged by its loss.

Acoustic speech and voice signal neural processing

Sophie. K. Scott
Institute of Cognitive Neurosciences, London, UK

In my talk I will outline how we can use functional imaging to investigate speech processing in the human brain, and identify the roles of hemispheric asymmetries, streams of processing and interactions with higher order language processes in the neural basis of speech perception. I will extend this to address how hearing brains process simulations of cochlear implants, and how we can identify the neural changes associated with adaptation to this novel form of speech. I will also show how a distributed system in the inferior frontal gyrus and angular gyrus are associated with individual differences in ability to understand these simulations. I will finish by considering some ways that function in this system might relate to speech perception tasks.

Role of transcription factors in the evolution of the visual system

Don Zack
Johns Hopkins Hospital, Baltimore, USA

Retinal development, function, and disease are, to a significant degree, controlled by the pattern of genes expressed by the cells of the retina. In an effort to better understand the mechanisms regulating photoreceptor gene expression, several groups, including our own, have been studying selected rod and cone enriched genes as model systems. Using a variety of approaches, some of the DNA regulatory elements and transcription factors important for rod and cone specific gene expression have been identified. Interestingly, it has been found that mutations in several of these transcription factors can lead to retinal degeneration in both mice and man. This talk will summarize some of these studies as well as present more recent data pertaining to the regulation of retinal gene expression during development and degeneration. In addition, some ongoing studies related to miRNA expression and alternative retinal RNA splicing during development will be covered.

Hair-cell function through evolution

Hernán López-Schier
Laboratory of Sensory Cell Biology & Organogenesis, Centre de Regulació Genòmica – PRBB, Barcelona, Spain

Mechanosensory hair cells show substantial similarities in their development and physiology across the evolutionary three. However, while their loss is irreversible in mammals, other vertebrates can recover hair cells after a damage of their sensory epithelia. In the lateral-line organs of aquatic vertebrates, for example, regeneration follows a choreographed set of steps. I will discuss that zebrafish neuromasts contain three distinct supporting-cell populations and each plays a unique role during hair-cell regeneration. Using quantitative three-dimensional multispectral live imaging, we have identified resident hair-cell progenitors as unipotent transient amplifying cells. Combined genetic and pharmacological analyses reveal a potential evolutionarily-conserved role of intercellular communications mediated by the Notch receptor in restricting the production of hair-cell progenitors from regenerative compartments. I will demonstrate the existence of resident bona fide hair-cell progenitors, and provide a detailed and comprehensive picture of the Notch-dependent spatiotemporal control of hair-cell regeneration. I shall also define a framework for further molecular interrogation of the regenerative process in vivo in an intact mechanosensory organ.

Development of the vertebrate retina

A. De Marzo[1], A. Comitato[1], C.G. Aruta[1], G.C. Demontis[2], and **V. Marigo**[1]
[1]Dept. of Biomedical Sciences, University of Modena and Reggio Emilia, Modena, Italy; [2]Dept. of Psychiatry, Neurobiology, Pharmacology and Biotechnology, University of Pisa, Italy

Development and differentiation of the retina are complex processes. Many of the components utilized as regulators of retinal development also participate in control of cell maintenance later in life. A better knowledge of these processes will provide us with a wealth of information necessary to construct strategies for cell protection or transplantation. Common and currently intractable diseases of the eye involve retinal degeneration generally leading to severe visual impairment and blindness. This group of diseases is characterized by progressive loss of rod photoreceptors. To develop therapeutic strategies requires full knowledge of the molecular signatures of normal and aberrant rod photoreceptors differentiation. An in vitro system able to reproduce rod differentiation and cell death in pathological conditions will allow us to perform molecular studies aimed at characterizing molecular events underlying rod cell differentiation and cell death. To this purpose we have developed a protocol for in vitro differentiation of rod photoreceptors. We derive retinal stem cells from the ciliary body of adult mice and grow them as neurospheres in minimal medium. Cells are then allowed to differentiate on an extracellular matrix substrate in the absence of serum and in the presence of differentiating factors. This protocol favors rod differentiation at the expenses to other retinal cell types. Expression of several rod-specific markers has been analyzed at the mRNA and protein level. Expression, subcellular localization and morphology suggest that the differentiation protocol allows a high percentage of cells to acquire a rod-like phenotype in vitro. Functionality of the cells has been assessed by patch-clamp recording after differentiation, electrophysiological data will be present. Finally, we showed that rod-like cells derived from animal models of Retinitis Pigmentosa undergo spontaneous apoptosis in vitro and therefore demonstrate to be a useful model for molecular studies of pathways activated during retinal degeneration.

Development of the vertebrate inner ear

Donna Fekete
Purdue University, West Lafayette, USA

The Wnts are a large family of secreted signaling proteins that mediate many processes in development. We have been using the chicken embryo as a model to study the role of Wnt-related genes during otic morphogenesis, sensory fate specification, axon guidance and hair cell planar cell polarity. We recently completed a comprehensive topological Wnt expression map of the inner ear from E4-E15. We find complex spatiotemporal expression patterns for many Wnts, Wnt receptors (Frizzleds) and Wnt inhibitor transcripts that can inform more functional studies. Evidence that Wnt/β-catenin signaling regulates a sensory fate choice between auditory and vestibular organs has led to a search for the endogenous Wnts that may influence this decision. In vitro assays for outgrowth of the statoacoustic ganglion have failed to show evidence that Wnt4 or Wnt5a, which are found adjacent to some inner ear sensory organs, act to repel axons. Finally, our interest in asking whether Wnts play an active role in polarizing the hair bundle have led us to manipulate one core planar-cell-polarity gene, *Vangl2*. Virus-mediated misexpression of this gene in the auditory organ suggests that, like fly bristle orientation, planar-cell-polarity information can be propagated unidirectionally to perturb bundle orientation in a non-cell autonomous fashion.

Genetic dissection of hindbrain commissures

Alain Chedotal
INSERM UMRS_968, Institut de la Vision, Department of Development, 17 rue Moreau, 75012 Paris, France

At all levels of the vertebrate nervous system, axons cross the midline to form commissural projections. In the visual system or the corpus callosum in the neocortex, the physiological importance of brain commissures is well established in part due to the "split-brain" studies of Roger Sperry and others. However, the function of commissural projections in the hindbrain and spinal cord is more difficult to assess. We imagined to genetically re-wire the hindbrain by forcing select commissural tracts to remain ipsilateral. By subsequently examining the cellular and behavioral consequence of these manipulations, we envisionned to broaden the current understanding of the function of specific commissures. We focused on Robo3, a vertebrate roundabout receptor involved in the formation of commissures in hindbrain and spinal cord. In humans, mutations in ROBO3 cause a rare syndrome called "horizontal gaze palsy with progressive scoliosis" (HGPPS)
The cause of horizontal gaze palsy, one of the two signature traits of HGPPS syndrome, is still unknown. In addition, a subset of HGPPS patients show an unexplained asymmetry of brainstem auditory evoked potentials and activation of their auditory cortex. To uncover the cause of these auditory and oculomotor deficits, we took advantage of multiple Cre transgenic lines to induce site-specific deletions of the Robo3 receptor. This led to the disruption of specific commissures in the sensory, motor and sensorimotor systems resulting in severe and permanent functional deficits. Surprisingly, although rerouted axons remain ipsilateral, they still project to their appropriate neuronal targets. We found that mice lacking commissures in rhombomeres 3 and 5 have, as described in HGPPS patients, abnormal eye movements and auditory brainstem responses. Therefore, *Cre;Robo3* lines represent good models for human syndromes, including Horizontal Gaze Palsy with Progressive Scoliosis (HGPPS).

Genetics of retinitis pigmentosa with a special focus on splicing factor genes

Shomi Bhattacharya
UCL-Institute of Ophthalmology, Bath Street, London, UK

Retinitis pigmentosa, a rod photoreceptor cell disease of the retina, is clinically and genetically heterogeneous with autosomal dominant (adRP), autosomal recessive (arRP) and x-linked (xlRP) modes of inheritance. So far 44 genes have been implicated in RP with at least 15 genes for the dominant form alone. This is expected given the unique and complex biology and function of the photoreceptor cell. The number of genes associated with RP is likely to increase given the number of patients / families in which mutation have still not been identified. This is also supported by the finding that animal models and functional studies have identified a number of highly relevant retinal genes that have so far not been implicated in RP.

Genetic linkage studies in large British adRP families allowed us to map a disease locus (RP11) to chromosome 19q. A positional cloning and bioinformatics based approach led to the cloning of the RP11 gene. Homology screening of protein databases revealed homology to proteins in yeast and drosophila involved in pre-mRNA splicing. Pre-mRNA splicing occurs by a two-step transesterification mechanism involving cleavage at the 5' and 3' splice sites and exon ligation. A large protein-RNA complex termed the spliceosome catalyses this reaction. Interestingly three other pre-mRNA splicing factor genes *PRPF8*, *PRPF3* and *PAP-1* have also been implicated in adRP. Identification of these genes revealed an entirely novel mechanism of photoreceptor degeneration distinct from those operating in other forms of RP where mutations have previously been identified in phototransduction proteins, structural proteins and transcription factors.

PRPF31 consists of 14 exons, spans approximately 18kb of genomic DNA and encodes a protein of 499 amino acids. It is involved in the recruitment of U4/U6.U5 tri-snRNP to the prespliceosomal complex. We propose that mutations in this gene lead to haploinsufficiency and due to the splicing stress only experienced in rod cells (due to the high expression of photoreceptor genes such as rhodopsin), having 50% wild type (WT) protein is not enough to cope with splicing in the rod cells. As a result perhaps required levels of protein production for the maintenance of rod outer segment ultrastructure and phototransduction is not achieved. Whereas in all other cell types in the body, 50% WT protein is perhaps enough to cope with the splicing requirement since none of the other cells have the same unique biology of extremely high turn over of proteins as in the rod cells. Hence, only rod-cell degeneration and RP results in patients with mutations in *PRPF31* and the other splicing factor genes including *PRPF3, PRPF8* and *PAP-1*.

MicroRNAs in the vertebrate inner ear

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MicroRNAs (miRNAs) are 17-24 nucleotide-long non-coding RNAs processed from transcripts of endogenous genes that regulate expression of protein-coding genes by inducing degradation of mRNA of target genes or by inhibiting translation. The nuclease Dicer1 is required for production of mature and functional miRNAs from pre-miRNAs. Their relevance to the inner ear has recently been emphasized by the discovery of miRNA mutations leading to deafness in humans and mice.
To perform a comprehensive study of miRNAs in the auditory and vestibular systems, we have and are taking several approaches. We identified miRNAs that may play a role in the mouse developing inner ear by combining miRNA transcriptome analysis and bioinformatics and determined the spatial and temporal expression patterns of a subset of these miRNAs. *Dicer1* was conditionally knocked-out in the sensory epithelia of the mouse inner ear, leading to impaired hearing. In order to optimize the search for miRNA targets, we combined a comparative transcriptomic and proteomic analyses with a miRNA screen of early post-natal cochlear and vestibular sensory epithelia derived from mice. An integration of these approaches is being used to elucidate the mechanisms underpinning miRNA regulation in the inner ear.

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Signal transduction in photoreceptors and related diseases

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The vertebrate retina contains two types of photoreceptors, rods and cones, that are specialized for high-sensitivity vision under dim light conditions, and high contrast, high-acuity daylight and chromatic vision, respectively. Derived from a common ancestor, rods and cones have evolved analogous signaling pathways involving isoforms of enzymes and structural proteins dedicated to their specific requirements. In fact proteins participating in the signal transduction pathway in rods and cones are mostly encoded by distinct genes that are specifically expressed in either rods or cones.
Signal transduction in photoreceptors can be divided in two main parts: 1) the phototransduction cascade in the outer segment including shut-off and regeneration cycles and adaptation processes, and 2) modulation and shaping of the hyperpolarization signal and synaptic transmission. Photoreceptor signal transmission is unusual in the sense that stimulation by light results in a membrane hyperpolarization and a subsequent tonic depression in glutamate release from a ribbon-type synapse.
The process of phototransduction has been intensively studied intensively and the identity and properties of the involved factors are well-characterized, at least in rods. It follows a unique GPCR signaling pathway that couples to a membrane-associated, cGMP-specific phosphodiesterase. cGMP serves as the principle ligand of photoreceptor CNG channels that mediate the dark current and close at low cGMP levels, creating the membrane hyperpolarization at sustained ATP-dependent cation efflux.
Genetic studies in hereditary retinal dystrophies have identified mutations in most of the genes that encode for the principal components of the phototransduction cascade. Since rods and cones mostly employ distinct isoforms encoded by different genes, those mutations impair primarily the function of either rods or cones. Yet rods and cones are physically and physiologically coupled and tighly interconnected. Therefore at the level of the neuroretina, an impairment of the rod phototransduction which eventually leads to rod degeneration will often also impair cone function and/or survival during the course of the disease. Most mutations in genes that encode for components of the phototranduction cascade behave recessive due to loss of function effects. A notable exception are mutations in the rhodopsin gene (RHO) which mostly causes dominant inherited Retinitis pigmentosa. In this case it seems that mutant rhodopsin either elicites ER stress and triggers UPR-dependent apoptosis or impair regular phagocytosis and lysosomal digestion of outer segments by the RPE that secondarily kills photoreceptors.
Modulation of the hyperpolarization signal and synaptic transmission in photoreceptors involve proteins that are recruited from the standard repertoire of neuronal ion channels, though the actual subunit composition may be specific or involve modulatory subunits with restricted expression patterns. These involve for instance voltage-gated calcium and voltage-gated potassium channels, and hyperpolarization activated CNG channels. Therefore only few retinal specific disorders are known that are caused by mutations in genes involved in that function. Those disorders (for instance CSNB or CDSRR) are usually milder and less progressive and can often be recognized by certain aspects in electroretinographic responses.

Mechanotransduction of mammalian cochlear hair cells

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Cochlear hair cells, the sensory receptors of the inner ear, transform sound evoked vibrations into electrical signals encoding the frequency and intensity of the acoustic stimulus. Displacement of their stereocilia hair bundle opens mechanoelectrical transducer (MT) channels, allowing the entry of K and Ca which in turn creates a depolarizing receptor potential in the hair cells. Although these channels are not yet molecularly identified, their biophysical properties have been extensively documented. They are none selective cation channels, highly permeable to calcium and with a large single channel conductance. A prediction of two channels per tip link, link connecting contiguous stereocilia, is predicted. Although evidence suggests the MT channels are near the tops of the stereocilia and are opened by force in tip links, the exact channel site remains controversial. Since MT channels are highly permeable to calcium, monitoring calcium entry with high speed confocal imaging showed a concentration of the channels to the bottom end of the tip links.

New insight towards the understanding of night blindness

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Night vision requires signaling from rod photoreceptors to adjacent bipolar cells in the retina. Mutations in the genes *CACNA1F*, *CABP4* and *CACNA2D4*, expressed at the synapses of the photoreceptor cells lead to a disruption of the ON and OFF bipolar cell response. This dysfunction is present in patients with X-linked and autosomal recessive incomplete congenital stationary night blindness (icCSNB). Mutations in the genes *NYX* and *GRM6*, expressed in ON bipolar cells, lead to a disruption of the ON bipolar cell response. This dysfunction is present in patients with X-linked and autosomal recessive complete congenital stationary night blindness (cCSNB). Both forms can be discriminated by standard full-field electroretinography (ERG). The incomplete type is characterized by both a reduced rod b-wave and substantially reduced cone response, while the complete type is associated with a drastically reduced rod b-wave response, but largely normal cone b-wave amplitudes. Although many cases of CSNB are caused by mutations in the above mentioned genes, in several patients the gene defect remains unknown. Animal models of human diseases are a good source for candidate genes, and we noted that a cCSNB phenotype present in homozygous *Appaloosa* horses is associated with downregulation of *TRPM1*. TRPM1, belonging to the family of transient receptor potential channels, is expressed in ON bipolar cells and therefore qualifies as an excellent candidate. Indeed, mutation analysis of 38 patients with CSNB identified ten unrelated cCSNB patients with 14 different mutations in this gene. The mutation spectrum comprises missense, splice-site, deletion, and nonsense mutations. We propose that the cCSNB phenotype in these patients is due to the absence of functional TRPM1 in retinal ON bipolar cells. Based on our studies we would like to propose that *TRPM1* is the most frequently mutated gene in autosomal recessive CSNB.

Mechanosensation in auditory sensory cells: what did we learn from deafness genes?

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Until recently the molecular mechanisms underlying the differentiation and functions of the auditory sensory hair cells were entirely unknown due to the paucity of these cochlear cells. The study of inherited deafness forms, whether syndromic or isolated, allowed to open this field of research. The presentation will focus on the contribution of the corresponding genes regarding the way in which the hair bundle, the mechanoreceptive structure of the hair cells, develops and works. We gained major insights thanks to the genes defective in Usher syndrome (sensorineural deafness associated with retinitis pigmentosa).
Evidence of colocalisation, direct in vitro interactions, and interdependence of localisation in the hair-bundle of all the proteins encoded by the Usher I genes (cadherin23, protocadherin15, harmonin, sans and myosin-VIIa) led us to include them in the same molecular complex underlying the composition of early transient hair-bundle links and their anchorage to the stereocilia actin filaments. Morphofunctional analyses of mouse mutants showed the critical roles of these early transient hair-bundle links in the early cohesion of the hair-bundle. Other links, the top connectors, proved to be essential for hair-bundle production of sound waveform distortions.
On the basis of the involvement of both cadherin23 and protocadherin15 in the composition of the tip-link which is believed to control the gating of the auditory mechanotransduction (MET) channel, we considered as well a possible role of the other Usher-1 proteins in the MET machinery. The involvement of Harmonin-b, a PDZ protein anchoring the tip-link to the actin filaments, in the MET adaptation process has been demonstrated. Preliminary indications also suggest the involvement of sans in the MET machinery.

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Lefèvre G, Michel V, Weil D, Lepelletier L, Bizard E, Wolfrum U, Hardelin J-P & Petit C (2008) A core cochlear phenotype in USH1 mouse mutants implicates fibrous links of the hair bundle in its cohesion, orientation and differential growth. Development 135, 1427-1437.

Michalski N, Michel V, Caberlotto E, Lefèvre GM, van Aken AFJ, Tinevez J-Y, Bizard E, Houbron C, Weil D, Hardelin J-P, Richardson GP, Kros C, Martin P, Petit C (2009) Harmonin-b, an actin-binding scaffold protein, is involved in the adaptation of mechanoelectrical transduction by sensory hair cells. Pflügers Arch-Eur J Physiol 459, 115-130.

Petit C, Richardson GP (2009) Linking genes underlying deafness to hair-bundle development and function. Nat Neurosci 12, 703-10.

Verpy E, Weil D, Leibovici M, Goodyear RJ, Hamard G, Houdon C, Lefevre GM, Hardelin J-P, Richardson GP, Avan P & Petit C (2008) Stereocilin-deficient mice reveal the origin of cochlear waveform distortions Nature 456, 255-8.

From rare to complex disease

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Complex disorders are by definition difficult to understand. Particularly in the context of ageing, they arise on a background of wide-ranging abnormalities at cellular and molecular levels and it is perhaps not surprising that finding effective therapies has proved to be such a challenge. We know that even with relatively simple monogenic conditions there can be wide ranging phenotypic variation from patient to patient. How can we understand a condition where many genetic and environmental events conspire to create a specific pattern of disease in a given individual?
One answer lies in harnessing the wealth of knowledge that single gene disorders have to offer. The first step is to recognise that in general there is probably not one single thing that fails in disorders such as AMD. We should therefore consider such conditions as systems disorders set in the context on an ageing eye. AMD then becomes, not a disorder of the photoreceptor, the RPE cell, the choriocapillaris or Bruch's membrane but a disorder of a system that comprises all these elements and more. Monogenic conditions then provide insights into how the system behaves rather than models for AMD. The distinction is important. Sorby's fundus dystrophy and a number of other conditions rightly focus our attention on dynamics of extracellular matrix turnover but AMD is not a TIMP-3 disorder. Stargardt's disease has much understanding of the toxicity of A2E but A2E is not necessarily central to the pathogenesis of AMD. The above approach will be expanded to consider other rare conditions in order to provide an overview of pathways that are likely to be perturbed in AMD.

Sensory deficits and disease mechanisms of Bardet-Biedl syndrome

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The ciliopathies are an expanding group of clinical disorders arising from dysfunctional cilia. Chief among these is the Bardet-Biedl syndrome comprising retinal degeneration, polydactyly, renal dysfunction and cognitive impairment. Recent studies have defined additional phenotypes such as hearing loss and olfactory defects. BBS therefore displays a spectrum of sensory deficits which overlaps with several other ciliopathies. Investigation of hearing loss in BBS mice has for example uncovered stereociliary bundle misorientation reminiscent of planar cell polarity mutants. We have shown that BBS4 and 6 interacts genetically with the core PCP protein Vangl2 and that BBS proteins are required for regulation of PCP signalling. BBS proteins have also been shown to be important in coordinating ciliary intraflagellar transport processes thereby potentially linking cilia function to PCP. Here I will discuss the sensory aspects of BBS and explore the relationship of these to cilia and PCP in particular.

Sensorial manifestations in syndromic ciliopathies

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The ciliopathies are an expanding group of inherited disorders due to the dysfunction of the centrosome /primary cilia complex. The photoreceptor cells are modified cilia and represent a target organ for clinical expression of genetic defects in cilia related genes. Indeed, a number non syndromic and syndromic retinitis pigmentosa have been recognized to be ciliopathies.
A high level of genetic heterogeneity has been identified for this group of retinal dystrophies highlighting the complex mechanisms underlying the impact on photoreceptor function. Moreover, the extra ocular clinical consequences show that the pathogenesis is reaching many cellular types and various tissue functions. A general clinical, with emphasis on the retinal phenotype, and genetic overview will be presented as well as the main pathogenesis mechanisms know to date.

Shaping the mammalian hearing organ by the planar cell polarity pathway

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The mammalian hearing organ, the organ of Corti, is suspended along the spiraled cochlear duct and consists of stereotypical arrays of uniformly oriented sensory hair cells. During development, postmitotic precursor cells of the organ of Corti undergo cellular rearrangement, resulting in cochlear extension and uniform orientation of hair cells. Recently studies indicate the involvement of a conserved genetic pathway, the planar cell polarity (PCP) pathway in regulating both cochlear extension and hair cell orientation. We and others also demonstrated that the ciliary genes are novel vertebrate PCP components and are required for cochlear extension and hair cell polarization.
We further characterized cellular behavior and the polarization of primary cilia and associated basal bodies in normal and mutant mice to dissect the cellular roles of primary cilia in PCP signaling in the cochlea. We visualized unidirectional extension of the organ of Corti during terminal differentiation and identified remodeling of cellular contacts that are indicative of cellular intercalation in the extension of the organ of Corti. Moreover, our data supports two downstream signaling pathways involving ciliary axoneme and cell adhesion or the basal body and the Usher complex for cochlear extension or hair cell polarity, respectively.

The Usher interactome

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Usher syndrome is the most common cause of hereditary deaf-blindness in man and clinically and genetically heterogeneous. Eleven loci are known for Usher syndrome, and the nine known genes encode proteins that belong to very different protein families. We and others have provided evidence for the existence of a protein network of Usher proteins at different subcellular sites in the retina and inner ear. The Usher protein complex is dynamic both in localisation and composition. In hair cells of the inner ear, Usher proteins are essential for normal development of the hair bundle and are part of the fibrous links that connect stereocilia and stereocilia and the kinocilium. In photoreceptor cells, Usher proteins are located in the connecting cilium the basal body and also in the periciliary region. Fibrous links connect this periciliary region and the connecting cilium and GPR98 is essential to form these links. Therefore, these links might be reminiscent of the links in the inner ear hair bundles. Usher proteins are also detected in the synaptic region of both hair cells and photoreceptor cells.
To further elucidate the pathogenic mechanisms of Usher syndrome, we searched for novel members of the Usher protein complex, also called Usher interactome, by screening yeast two-hybrid retinal cDNA libraries. This revealed several interaction partners for the intracellular region of USH2A, including the centrosomal protein NINL. This protein was found to be a molecular link between Usher syndrome and two other disorders with retinal degeneration, Bardet-Biedl syndrome and Leber congenital amaurosis. This suggests common pathogenic mechanisms in these disorders. Furthermore, the physical and genetic interactions between proteins/genes involved in Usher syndrome and Bardet-Biedl syndrome suggest a role for the Usher interactome in the establishment of planar cell polarity, with a central role of the cilia.

Mutation spectrum of rhodopsin mutations in French autosomal dominant rod-cone dystrophy patients and their phenotype/genotype correlation

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Phenotype/genotype correlation of retinal dystrophies is essential to deepen our knowledge in pathophysiological mechanisms and prepare patients for future therapies. Furthermore, little is known in gene involvement, the nature of mutations and frequencies in the French population. Rhodopsin (*RHO*) has been one of the first genes implicated in rod-cone dystrophies, also called retinitis pigmentosa (RP) and is the most commonly mutated gene in autosomal dominant (ad) RP in the US. For these reasons, we decided to conduct a systematic study of *RHO* in a clinically well-characterized adRP cohort of patients from 2 different clinical French centres, Paris and Montpelier. Detailed phenotypic characterization was performed in these patients including precise family history, best corrected visual acuity, slit lamp examination, kinetic and static perimetry, full field and multifocal ERG, fundus autofluorescence imaging and OCT. For genetic diagnosis, genomic DNA isolation and sequencing was performed by standard methods. From 79 families investigated 16.5% revealed a *RHO* mutation. While three unrelated families showed each a novel missense mutation (p.Leu88Pro, p.Met207Lys and p.Gln344Pro), ten unrelated families showed previously published mutations: two revealed the p.Asn15Ser exchange, one revealed the p. Leu131Pro substitution, two other patients the p.Arg135Trp substitution, one patient the p.Ala333fsX22 frameshift mutation and four patients the known p.Pro347Leu exchange. All mutations co-segregate with the phenotype within a family and the novel mutations were not identified in a control population. Our studies revealed that the prevalence of *RHO* mutations in French autosomal dominant rod-cone dystrophy patients is less prominent than reported in United States or United Kingdom, but is in accordance with other studies from Spain, Germany, Italy and Southern France. Most of the changes identified herein reflect recurrent mutations within which p.Pro347Leu substitution is the more prevalent. Nevertheless, almost a quarter of the changes are novel indicating that, although *RHO* was the first gene implicated and probably the most studied gene in RP, it is still relevant to perform mutation analysis in the coding exons of *RHO* to detect novel changes. Clinical characterization of the patient cohort outlines the phenotypic variation associated with rhodopsin mutations with both restricted and generalized retinal involvement. Bioinformatic analysis of mutation consequences on protein function gives further information on pathophysiologic mechanism. Our detailed phenotype-genotype analyses in all family members available deliver the basis for therapeutic approaches in those families.

The role of connexins in sensorineural hearing impairment

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Connexins are the integral membrane proteins that compose the intercellular channels of vertebrate gap junctions. The connexin protein family in humans comprises 22 members, several of which have been implicated in genetic disorders as diverse as cataracts, peripheral neuropathies, genodermatoses, oculodentodigital dysplasia or Pelizaeus-Merzbacher-like disease. In particular, mutations that affect the genes encoding the connexin family members Cx26 and Cx30 at the DFNB1 locus are the single major cause of inherited hearing impairment in Caucasians and contribute a significant proportion of cases in other populations worldwide. Moreover, two other connexins, Cx31 and Cx29, have been implicated in deafness in humans and mice, respectively. In spite of this, the roles of connexins in the pathophysiology of hearing are not yet fully understood. This presentation will summarize the most relevant data on the genetics of hearing impairment due to mutations in genes encoding connexins. Specifically, the known functions of connexins in the physiology of hearing (roles in gap junction-mediated intercellular communication and paracrine signaling), their distribution in the mammalian inner ear, and our current knowledge about the pathogenesis of DFNB1 hearing impairment (gleaned both from the investigation of human patients and from the analyses of murine models lacking specific connexins) will be discussed.

Molecular conservation of eye and ear development

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Molecular development of the neurosensoy parts of the ear is driven by a small set of bHLH genes that have been shown to be essential for sensory neurons (Neurog1, Neurod1) and hair cell development (Aoth1). Many more genes have been shown to modify the function of these bHLH genes by altering the number and distribution of hair cells or regulating the survival of sensory neurons. We will present recent work that demonstrates that this simple scheme we and others presented 10 years ago needs to be revised by more realistic interaction of these three bHLH genes to define both neuronal and hair cell differentiation. For example, neuron differentiating bHLH genes are also expressed in subsets of hair cells and their absence either causes loss of hair cells or alteration in their phenotype. For example, loss of Neurog1 results in near complete loss of saccular hair cells and this relates to a clonal relationship as proposed 10 years ago. Absence of Neurod1 results in altered expression profiles of hair cell differentiating genes and changes the ratio and distribution of the two cochlear hair cell types, the inner the outer hair cells. In addition, Neurod1 is needed to suppress upregulation of the hair cell differentiating gene Atoh1 in sensory neurons and conversion of these cells into hair cells. Interestingly, those cells undergo a epithelial transition and form vesicles inside the remaining ganglion. Our data on bHLH genes emphasize a complexity of this principal regulators of neurosensory differentiation in the ear that will be take into account in future studies of modifiers. They also provide the rational basis for novel therapeutic approaches.

Function and dysfunction of photoreceptor ribbon synapses

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The photoreceptor ribbon synapse, the first station in signal transfer in the visual system, belongs to the most efficient and complex chemical synapses in the CNS. Photoreceptor ribbon synapses transmit signals over a dynamic range of several orders of magnitude in light intensity, and they continuously adjust their synaptic output to changing inputs. To accomplish this level of performance, a highly specialized presynapse is required. Functional defects at the photoreceptor ribbon synapses – so-called synaptopathies – can impair seeing ability up to and including complete blindness. Despite their central role in visual signal transfer little is known about the structure and function of the photoreceptor ribbon synapses. From our studies on mouse mutants with loss-of-function mutations in key presynaptic proteins we expect to gain a detailed molecular understanding of photoreceptor ribbon synaptic structure and function – the basis for a better understanding of the physiology and pathophysiology of this unique synapse.

Synaptic physiology of cochlear hair cells

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Mechanosensory hair cells of the mammalian cochlea convert acoustic energy into bioelectrical signals for neuronal processing. Glutamate release from ribbon synapses in hair cells activates AMPA-type receptors to excite type I and type II afferent neurons. Using gigaohm-seal 'whole-cell' recording, our laboratories (P. Fuchs and E. Glowatzki) have established several features (expected and unexpected) of afferent synaptic signaling in the cochlea. The Glowatzki lab continues to explore the contributions to, and limitations imposed on, type I afferent signaling by vesicular transmitter release from the inner hair cell ribbon synapse. We have just begun to learn about synaptic transfer onto type II afferents, although their 'low throughput' contact with numerous outer hair cells dictates a role distinct from that of the singular inner hair cell, type I afferent connection. In addition to these afferent pathways, medial-olivocochlear (MOC) efferents project from the brainstem to contact outer hair cells, while lateral-olivocochlear efferents synapse on type I afferent dendrites beneath inner hair cells. A. Elgoyhen established that the hair cell AChR includes two subunits, α9 and α10, and in collaboration we have begun exploring the functional consequences of specific alterations in this ligand-gated calcium channel. Several features of hair cell inhibition remain unresolved, including the relative contribution of calcium influx versus release from internal stores, mechanisms of synaptic plasticity, and the regulation of potassium channel function at these unusual inhibitory synapses. This presentation will summarize recent advances in cochlear synaptic physiology.

Defense against oxidative stress and redox signaling

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Reactive oxygen species (ROS) are formed from metabolism or during oxidative stress. Thioredoxin and glutaredoxin systems (1,2) are major disulfide reductases which have numerous roles in protection against ROS as well as redox signaling. Thioredoxin (Trx) acts as the cells major protein disulfide reductase together with NADPH and the selenoenzyme thioredoxin reductase (TrxR). The thioredoxin system in cytosol/ nucleus comprising Trx1 and TrxR1 serves as anelectron donor for ribonucleotide reductase, methionine sulfoxide reductases and peroxiredoxins (thioredoxin peroxidases) and are upregulated by oxidative stress. The corresponding mitochondrial enzymes are crucial for control of ROS from the electron transport chain together with glutathione (GSH) and glutathione peroxidases. Thioredoxin iself is subject to redox regulation by modification of its three structural SH-groups (3) and can be secreted both as full-length protein and as a truncated form called Trx80, which acts as a growth factor for monocytes. Trx1 itself has cocytokine activity and the plasma levels of Trx1 reflect inflammation and oxidative stress. Glutaredoxins (Grx) are glutathione-dependent oxidoreductases catalyzing GSH-dependent disulfide reduction and deglutathionylation of proteins. Human Grx2 has splice forms and exists as Grx2a in mitochondria where it has an antiapoptotic effect. In contrast to Grx1 which can also be found in plasma, Grx2 is confined to mitochondria and present as an inactive dimeric holoenzyme containg a 2-iron 2 sulfur complex (4). Ebselen is a synthetic selenazol drug with GSH peroxidase and anti-inflammatory activity. Ebselen is an exceptionally fast oxidant of reduced Trx and a direct substrate of mammalian TrxR and a promising drug to combat oxidative stress diseases (5).

References:
1. Lillig, C.H. and Holmgren, A. Thioredoxin and related molecules: from biology to health and disease. Antioxid. Redox Signal. 9, 25-47, 2007.
2. Lillig, C.H., Berndt, C., and Holmgren, A.: Glutaredoxin systems. Biochem. Biophys. Acta 1780,1304-1317, 2008.
3. Hashemy, S.I., and Holmgren, A: Regulation of the catalytic activity and structure of human thioredoxin1 via oxidation and S-nitrosylation of cysteine residues. J. Biol. Chem.283, 21890-21898, 2008.
4. Lillig, C.H., Berndt, C., Vergnollé, O., Lönn, M.E., Bill, E. and Holmgren, A.: Characterization of human glutaredoxin 2 as new iron-sulfur protein: a possible role as redox sensor. Proc. Natl. Acad. Sci., USA, 102, 8168-8173, 2005.
5. Zhao, R., Masayasu, H. and Holmgren, A.: Ebselen: a substrate for human thioredoxin reductase strongly stimulating its hydroperoxide reductase activityand a superfast thioredoxin oxidant. Proc. Natl. Acad. Sci. USA, 99, 8579-8584, 2002.

The RdCVF signaling

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We have identified a novel trophic signaling in the retina that involves a novel family of proteins, the Rod-derived Cone Viability Factors (RdCVFs) that share sequence homology with the redox-controlling enzymes thioredoxins. The potential for these factors to prevent the loss of central vision in patients suffering from retinitis pigmentosa (RP) was demonstrated by delivering RdCVF in the P23H transgeneic rat model of RP. We demonstrate now that this novel signaling is involved in the maintenance of photoreceptors in the adult animal by studying the visual deficits of the mice carrying an inactivation of the *Nxnl1* or *Nxnl2* genes, encoding respectively for RdCVF and RdCVF2. Interestingly the *Nxnl1-/-* retina is hypersensitive to oxidative stress. The second product of the *Nxnl1* gene, the nucleoredoxin-like protein RdCVFL interacts and controls the level of phosphorylation of the TAU protein in the retina in agreement with a dual function for these genes which each encode for one enzymatic product sensing the redox potential RdCVFL and a trophic protein RdCVF with integrated functions. We demonstrate that the *Nxnl1* and *Nxnl2* genes are part of an endogenous redox signaling that couples the amount of oxidative stress to the function and the survival of cone photoreceptors.

Neuroprotection in rod-cone dystrophies: from bench to bedside

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In recent years, besides the discovery of numerous novel mutations and disease-causative mechanisms, major advances in therapy have opened the field of retinal degenerations research towards a new era. These include the striking success of gene therapy in Leber's Congenital Amaurosis, the partial but obvious rehabilitation potential afforded by retinal prostheses, the emerging field of optogenetics, and the potential of stem cells, in a more remote future. Such progresses lead to even more emphasis on the urgent need of large scale genotyping methods, as well as accurate phenotyping tools in order to define the target population for both efficient clinical trials and eventual approved therapies. The definition, characterization and validation of markers (biologic and morpho-functional) is a major challenge and opportunity for the coming years. This holds especially true for neuroprotective strategies. Neuroprotection in degenerative neuronal diseases is often viewed as a frustrating field as a consequence of both the poor characterization of the patients enrolled in trials (length and stage of the disease), the lack of accurate outcome measurements, the length and cost of trials, contrasting with the very low rate of success. Recent results from several trials in retinal degenerations such as ECT-based delivery of CNTF, modulation of the visual cycle, and the current conduct of several phase II or III studies testify that academic teams, industrials and clinicians still strive to validate this approach.
The recent developments in high resolution retinal imaging offer unprecedented opportunities to define more fitting target populations and efficacy markers for future studies.

Gene therapy for retinal dystrophies

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Given the advances of gene therapy studies to cure RPE65-derived Leber Congenital Amaurosis (LCA) (clinical trials phase I), it is of prime importance to optimize gene transfer for a gene replacement strategy and to develop a generic approach for patients not eligible for a gene replacement approach. In a first series of experiments, we examine how cones can be rescued in different mutant contexts of *Rpe65*. Consequently, we evaluated the effect on retinal activity and cone survival of lentivirus-mediated gene therapy in the R91W knock-in mouse model expressing the mutant $Rpe65_{R91W}$ gene. In a second work, we investigated the cell death pathway in the Rd1 (Pde6b mutation) to potentially identify a target for drug or gene therapy to slow down retinal degeneration.
An HIV-1-derived lentiviral vector (LV) expressing either the *GFP* or the mouse *Rpe65* cDNA under the control of a 0.8 kb fragment of the human *Rpe65* promoter (R0.8; LV-R0.8-RPE65) or LV-R0.8-*GFP* were tested into 5-days-old (P5) or 1 month-old R91W mouse retina. Functional and morphological retinal rescues were investigated at 4 months of age. Increased light sensitivity was detected by ERG and pupillary light responses in animals injected with LV-R0.8-RPE65 at both P5 and 1 month compared to controls. Histological analysis showed improved expression of cone markers and cone outersegment morphology. Furthermore, the density of cones in the region of RPE65 delivery after treatment at P5 reached the wild type level. However, before injection at 1 month of age, only a fraction of the cones (40% of the number found in WT animals) in the $Rpe65^{R91W/R91W}$ mice expressed cone transducin, this fraction increased to 64% after treatment. Moreover, these cones appeared normal.
We show that lentivirus-mediated *Rpe65* gene transfer is very efficacious in early treatments and still efficient during the course of cone degeneration. Moreover, the treatment at 1 month shows a regenerative process of the diseased cones. Thus patient suffering from R91W mutation might benefit from a prolonged therapeutic window.
For many forms of Retinitis pigmentosa the mutant gene is unknown or the gene function is not understood. We envisaged the possibility to alleviate the course of the severity of the disease by interfering with the process of cell death induction. In several neurodegenerative diseases the reactivation of cell cycle proteins is a key event that precedes neuronal apoptosis. We asked whether a similar phenomenon occurs in Rd1 mice, a model of retinitis pigmentosa widely used to study photoreceptor (PR) loss. We used different knockout mouse models to reveal whether proteins involved in the cell cycle regulation are responsible for photoreceptor loss in the Rd1 mouse. Histological and electroretinogram analyses were performed to evaluate the retina integrity.
At P12, an early stage of the disease, Rd1 mice displayed an increased expression of CDK4 and CDK2 among PR nuclei. PRs also undergo DNA synthesis. At P12, the polycomb protein Bmi1 was expressed in virtually all the nuclei in the inner and outer nuclear layer of both wild-type (WT) and Rd1 mice. Bmi1 promotes cell cycle progression via the repression of tumor suppressor genes. We reasoned that Bmi1 deletion could impede the aberrant CDK reactivation that characterizes neuronal apoptosis and may therefore delay retinal degeneration. We compared the histology of WT, Rd1 and Rd1;Bmi1-/- and observed the presence of 7 rows of PRs in Rd1;Bmi1-/- mice at P33, while Rd1 littermates displayed a single scattered row of PRs. ERG recordings revealed the ability of Rd1:Bmi1-/- retinas to respond to

light stimuli. Both DNA synthesis and CDK4 were strongly decreased in Rd1;Bmi1-/- mice, respectively by 70% and 50% as compared to Rd1 littermates.
In conclusion, our data show for the first time a mechanism of retina degeneration involving a reactivation of the cell cycle proteins that precedes PR death in Rd1 mice and reveal that the partial inhibition of cell cycle re-entry strongly delays PR loss. The Bmi1 pathway is in consequence an interesting target for drug and gene therapy to delay retinal degeneration.

Targeted expression of light sensitive pumps and channels restores visual function in animal models of Retinitis Pigmentosa

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In my talk I will show that the targeted expression of light gated channels or pumps ("optogenetic tools") in strategic cell types in different models of Retinitis Pigmentosa can restore activity to both the ON and OFF retinal channels. Resensitized cells drive sophisticated retinal circuit functions including directional selectivity, activate cortical circuits and mediate visually guided behaviours. Our results demonstrate that, despite the diverse genetic origin of Retinitis Pigmentosa, the targeted expression of a single gene can restore significant functionality to the visual system following degenerative changes.

Electronic retinal implants

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Electronic retinal implants aim at restoring a useful vision in patients following photoreceptor degeneration by stimulating the remaining retina with an electronic device. Indeed, photoreceptors can degenerate in different retinal diseases like retinitis pigmentosa and age macular degeneration. In retinitis pigmentosa for instance, rod photoreceptor degeneration often results from a mutation in a gene specifically expressed in these photoreceptors. The loss of rod photoreceptors leads to cone photoreceptor degeneration. The neuronal cell loss also affects the two other neuronal layers in the retina. However, even in the most advanced stages of the diseases, one fourth of retinal neurones remains after the complete loss of photoreceptors. The concept of electronic retinal implants relies on the stimulation of these remaining neurones to reintroduce some information into the visual system. Different clinical studies have demonstrated this concept. The electronic device can be implanted either on the vitreous side (epi-retinal implant) or in the subretinal space at the original position of photoreceptors (subretinal implant). Visual perceptions were first generated with epi-retinal implants. Patients could follow light targets, identify white object on a black background, recognize letters and walk along lines. However, some groups have suggested that applied currents would exceed safe limits. Subsequently, subretinal implants were also found to produce light perception, to allow light target detection and object identification. Recently, a patient with a subretinal implant in the central area was able to read a four letter words. In fact, psychophysic experiments had shown that text reading or locomotion in a complex environment would require visual perception with at least 600 independent pixels.
The presentation will illustrate these studies and describe recent investigations on diamond biocompatibility and on a small animal model of retinitis pigmentosa.

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Electrical stimulation of the human cochlea, auditory brainstem and midbrain

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Cochlear implants (CI) and auditory brainstem implants (ABI) have been successful beyond our wildest dreams. Most patients with CIs and some with ABIs can converse on the telephone with little difficulty and a few even enjoy music. The original approaches to prosthetic hearing failed to take into account the powerful pattern recognition capabilities of the brain. Individual differences in performance with ABI devices suggest the existence of multiple processing subsystems in hearing. Physiological and perceptual evidence suggest that the normal auditory system separately processes global envelope information and fine temporal and spectral information. Although the normal brain merges the outputs of these multiple systems together seamlessly, the optimization of prosthetic hearing devices requires that we better understand these different processing modes and the differential effect of hearing pathologies on these systems. While speech can be recognized with coarse envelope information, fine temporal and spectral structure is critical for musical pitch and for speech recognition in noise. In this talk I will review the progress with CI, ABI, and midbrain implant devices, discuss what we have learned about the brain's pattern processing power in hearing, and speculate on how sounds are processed in the brain and its implications for the design of future prosthetic devices.

Development and regeneration of the inner ear

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Generating the correct number and type of cells, in the correct ratio and spatial relationship is required for normal functional development of complex tissues. However, the mechanisms coordinating cell proliferation with growth and differentiation to achieve correct organ size and function remain a poorly understood aspect of embryonic development and postnatal regeneration. In this presentation, I will discuss our work on the molecular basis for the timing of cell cycle exit in the embryonic inner ear, and how this is coordinated with differentiation to produce the correct number of hair cell and supporting cell precursors to build a functional organ of Corti. In the second part of my talk I will discuss the mechanisms that govern cell fate decisions between sensory hair cells and the various supporting cell types in the developing organ of Corti. Specifically, I will focus on the role of Notch-dependent and Notch-independent mechanisms governing Hes and Hey gene expression, and their potential role in the maintenance of cell fate during subsequent maturation. Finally, I will discuss the potential role of these developmental processes in the failure of regeneration in the mammalian inner ear.

In vitro generation of hair cells from embryonic and induced pluripotent stem cells

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Our inner ear harbors about 15,000 cochlear and about the same number of vestibular sensory hair cells, which are the mechanoreceptors of our senses of hearing and balance. Because of their paucity, molecular studies on hair cells have been limited, and consequently, the inner ear shelters the last of our senses for which the molecular basis is unknown. Aside from being rare, hair cells are also sensitive to mechanical and chemical insults. Acoustical overstimulation, chemotherapy and aminoglycoside drug side effects, in addition to the effects of aging and the increasingly noisy environment contribute to the deterioration of hearing over time. As a result, hundreds of millions of patients worldwide are permanently debilitated by hearing loss and balance problems. The main reason for the permanence of these chronic disorders is the fact that mammalian cochlear hair cells do not spontaneously regenerate and that the limited regeneration observed in the vestibular system is inadequate to restore function. The main goals of our study were to employ principles of early embryonic development and otic induction to generate a population of otic progenitor cells capable of differentiating into mechanosensitive sensory hair cells. Embryonic stem (ES) and induced pluripotent stem (iPS) cells isolated from an identical murine model were used in parallel to show that both pluripotent cell types differentiate along the otic lineage without major differences. We identified conditions that promoted the differentiation of otic progenitors into hair cell-like cells that expressed multiple marker genes and displayed protrusions that are highly reminiscent of stereociliary hair bundles. Finally, we were able to show that ES and iPS cell-generated hair cell-like cells were responsive to mechanical stimulation and that these responses displayed transduction currents and adaptation reminiscent of hair cells.

From gene to drug design: mitochondrial deafness and aminoglycoside ototoxicity

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Mitochondrial rRNA mutations A1555G and C1494U are associated with deafness and hypersusceptibility to aminoglycoside antibiotics. Aminoglycosides are a class of structurally related antibiotics which interfere with decoding by binding to the A-site of small subunit rRNA. While these antibiotics preferentially target prokaryotic ribosomes, they are associated with significant ototoxicity. Despite recent advances in X-ray crystallography, little is known about the molecular mechanisms by which aminoglycosides result in toxicity and mutant mitochondrial ribosomes in disease.
We have established an experimental model for the study of eukaryotic A-site function by transplanting the eukaryotic small subunits' *rrn* decoding A-site into bacterial ribosomes. Hybrid ribosomes with the eukaryotic cytoplasmic ribosomes' decoding site were largely resistant to aminoglycoside action, while the mitochondrial-bacterial hybrid ribosomes showed a pattern of drug susceptibility to various aminoglycosides which correlated with their relative cochleotoxicity. Sequence alterations corresponding to mitochondrial A1555G and C1494U mutations significantly increased ribosomal susceptibility to aminoglycoside-induced mistranslation. We also found that the pathogenic mt rRNA mutations A1555G and C1494T by themselves decrease the accuracy of translation, suggesting misreading of the genetic code as an important mechanism in disease pathogenesis. Modelling the mitochondrial decoding site on available bacterial X-ray structures allowed us to develop a structure-function based hypothesis addressing the molecular mechanism of mutation-mediated misreading.
Having established the link between aminoglycoside toxicity and malfunction of the mitochondrial ribosome, we wished to address the question whether it is possible to redirect drug-target interaction, so as to develop more selective aminoglycoside derivatives. We used a set of genetically engineered ribosomal mutants to guide a step-by-step synthesis of novel aminoglycoside compounds. In comparison to available aminoglycosides the series of aminoglycosides synthesized have largely lost their antimitoribosomal activity, while retaining activity for the bacterial ribosome. In addition, the increased susceptibility of deafness hybrid ribosomes to aminoglycoside-induced malfunction of protein synthesis is almost absent for this series of compounds. These results testify to the feasibility of a combined genetic engineering/chemical synthesis approach to successfully alter drug-target interaction towards increased selectivity of aminoglycoside compounds.

Rehabilitation: reading training in patients with Stargardt`s disease - a randomized and controlled study

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Background: Stargardt`s maculopathy causes a central scotoma, which leades to reading disability, which often endangers their educational or professional life. We examined two training methods for optimizing reading ability in patients with central scotoma due to juvenile maculopathy with already established eccentric retinal fixation locus and optimal use of low vision aids.
Method: 36 patients were randomized into two groups: Group 1 received training to read during single-word presentation (rapid serial visual presentation, RSVP) with elimination of eye movements (n=20), Group 2 received training to optimize reading eye movements (sensomotoric training SM, n=16). Training was performed for 4 weeks, ½ hour per day and five days a week. Reading speed (page reading aloud) was measured before and after training. Eye movements during silent reading were recorded before and after training in 11 patients.
Results: Median reading speed was 83 words per minute (wpm) (interquartile range 74-105 wpm) in the RSVP training group and 102 (interquartile range 63-126 wpm) in the SM group before training and increased significantly to 104 (interquartile range 81-124 wpm) and 122, respectively (interquartile range 102-137 wpm; p=0.01 and 0.001) after training, i.e. patients with RSVP training increased their reading speed by a median of 21 wpm, while it was 20 wpm in the SM group. There were individual patients, who benefited strongly from the training. In the RVSP group, increasing reading speed correlated with decreasing fixation duration (r= -0.75, p= 0.03), whereas in the SM group, increasing reading speed correlated with a decreasing number of forward saccades (r=-0.9, p=0.01).
Conclusions: Although the median effect of both training methods was limited, individual patients benefited very much. Each training method indicates a specific effect on reading strategy: the RVSP method reduces fixation duration, the SM method decreases the number of forward saccades. Patients can apply their newly learned reading strategy in the natural reading situation, e.g. in page reading without special presentation of the text. Improvement of reading performance is important for patients` re-integration to educational or professional life and their quality of life in general.
For future studies, based on their eye movement pattern at baseline, patients can be selected and assigned to the appropriate training method. These results can be used as a basis for further improvement of training methods for optimizing reading performance in patients with a central scotoma.
Conclusion: Preconditions of a training effect are the optimal adaptation of low vision aids and the motivation of the patients.

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Patients' expectations

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Inherited retinal degenerative diseases are the main cause of childhood blindness. Very often they are combined with hearing loss presenting thus a double-sensory handicap. This means blindness is not only added by a hearing loss, this double - sensory handicap consists by itself a severe disability because each times the compensatory senses are impaired. Also people presenting „only“ at the beginning a visual handicap will most probably be confronted in their later life with this double-handicap due to the most probably inevitable hearing loss due to advanced age. Furthermore, new genetic results showed that a number of cases of Retinitis pigmentosa were due to a defect in usher 2 without these people presenting a marked hearing loss in their younger age.
Patient expectations are high: Of course every body would love to have vision restored. However confronted to an inherited retinal degeneration we get more modest or better said realistic: Slowing down the disease of get it stopped would be an excellent result by itself. Some therapies being on the horizon, the most prevalent expectations are the following:
- Access to early and correct diagnosis
- Access to genetic diagnosis whenever possible and wished by the patient
- Access to the best treatment modalities available
- Access to rehabilitation to guarantee social integration and a livelihood in dignity
- Research to be increased in order to find the cause, the missing genes, and therapeutic approaches to treat these rare diseases
- Centres of excellence for these rare diseases throughout Europe guaranteeing all European citizen access to the correct diagnosis, treatment and rehabilitation.

Patient expectations – the improvement of the quality of life of patients suffering from hearing impairment

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FAUN-STIFTUNG, Nürnberg, Germany

Over recent years the patient community in Europe has seen encouraging advances, accelerated scientific discoveries and promising options for therapies in the field of auditory research and developments. In this area, especially for those with disabilities due to defects in both sensory organs e.g. Usher syndrome, whether hereditary or not, the burning question remains, is there a chance, a hope to develop a therapy or cure, not one day in the distant future but as soon as possible. We feel in accordance with the UN Resolution that we have a right to live a life without the limiting burden of our disabilities.
We can see an increasing understanding of the sensory processes, basic molecular mechanisms underlying the physiology of the inner and outer ear hair cells, in identifying the genes and the mutations associated with a loss of hearing and seeing, in the development of new preventive and therapeutic tools. This is centered around gene therapy, stem cell research, the development of neurotropic factors, the technical advances in Cochlear Implants and other relevant areas. The challenges are multi-faced and success will primarily depend on the cooperation of interdisciplinary teams including patient participation. We as persons with disabilities expect "… to be active research partners – not passive guinea pigs, they are expected to become enlightened research supporters, or research managers, or researchers themselves, participating in scientific conferences like this one." (Anne Fagot-Largeault, Collège de France).
We are more than ready to cooperate and support your efforts actively.

Short talks

RanBP2-mediated neuroprotection is associated with the modulation of functionally diverse but linked pathways in response to oxidative stress

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Oxidative stress is a deleterious stressor associated with a plethora of diseases and aging manifestations. Yet, few factors promoting neuroprotection of photosensory neurons against light-elicited oxidative stress are known. Here, we present that deficits in the pleiotropic and mosaic protein, Ran-binding protein-2 (RanBP2), suppresses the age- and light-induced apoptosis of photoreceptors. Haploinsufficiency of RanBP2 and light-oxidative stress have a selective effect on protein homeostasis by down-regulating factors, such as Ran GTPase and ubc9/Ube2i, and blunted the up-regulation of a set of orphan nuclear receptors in the retina, whereas in the retinal pigment epithelium (RPE), such changes were not observed. These effects are also complemented by a genotype-independent increase of the levels of chaperones and mitochondrial components in the retina. Among the nuclear orphan receptors affected by RanBP2, we identified an isoform of Nr2f1/COUP-TFI as the only receptor stably associating in vivo with RanBP2. The downstream effects of insufficiency of RanBP2 cause a decrease of M-cone photoreceptors and a light-dependent decrease of ubiquitylated substrates in the retina. In the RPE, insufficiency of RanBP2 suppresses the light-dependent increase of lipophilic deposits, and it causes divergent effects in the accumulation of free cholesterol and fatty acids despite the genotype-independent increase of light-elicited oxidative stress in this tissue. Thus, the data support that insufficiency of RanBP2 causes the tissue-selective modulation of the expression of functionally diverse but linked pathways in response to oxidative stress and these effects may account for the neuroprotection of photosensory neurons to light damage.

Brn3a, Brn3b and Brn3c define distinct populations of Retinal Ganglion Cell Types

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In the vertebrate retina, the three members of the Brn3 family of POU-domain transcription factors are expressed exclusively in retinal ganglion cells (RGCs). Here we dissect the roles of the Brn3a, Brn3b and Brn3c genes in mouse RGC development and function using targeted conditional alleles permitting the visualization of individual wild type or mutant RGCs. Based on differences in dendritic arborization and axon brain projections, we find that the three Brn3s are expressed in overlapping but not identical population of RGC cell types. The loss of Brn3a alters the pattern of dendritic stratification, with little or no change in central projections. In contrast, loss of Brn3b leads to the loss of ~70% of RGCs, disorganized RGC axons in the eye and brain, and the loss or dysfunction of central projections that severely compromises non-image forming vision, including the optokinetic response, the pupillary light response, and photoentrainment. The striking differences between Brn3a and Brn3b mutant phenotypes imply that these highly homologous transcription factors play distinct roles in the genetic program that defines RGC identity. These findings offer potential tools for describing and manipulating specific RGC cell types and therefore visual channels and modalities.

Mitochondrial capacity as a biomarker of photoreceptor cell stress and neuroprotection

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Purpose:
Recent studies suggest that a gradual loss of metabolic capacity is common to photoreceptor cell pathologies. Typically, the loss in ability to maintain ATP synthesis flux arises from oxidative stress, increased calcium or swelling of the mitochondria, causing mitochondrial dysfunction. It is known that oxidative stress and calcium overload contribute to the pathology of Retinitis Pigmentosa and we hypothesize that these stressors diminish mitochondrial ATP production and respiratory capacity to cause retinal cell dysfunction and degeneration. On the flip-side, agents that increase mitochondrial respiratory capacity could be promising neuroprotective agents.
Methods:
To induce calcium or oxidative stress, 661W photoreceptor cells were treated with calcium ionophore A23187, phosphodiesterase inhibitor IBMX, oxidants tert-butylhydroperoxide or pro-oxidant paraquat. Metabolic responses were assessed from changes in extracellular acid release (ECAR) and oxygen consumption (OCR) measured with a Seahorse Biosciences extracellular flux analyzer. Maximal mitochondrial capacity was measured as the increase in OCR following uncoupling with FCCP protonophore in the presence of oligomycin, an ATPase inhibitor. Cell viability was assessed from dye exclusion assays. A 50,000-member library of agents from the DIVERSet collection (ChemBridge) were screened for their ability to protect cells from calcium-induced cell death. Lead candidates were tested in the extracellular flux assay and retina-RPE organ cultures of rd1 mice.
Results:
(1) Basal OCR and ECAR and maximal mitochondrial capacity were greatly impaired after addition of stressors. Changes in OCR and ECAR measured 45 min post-treatment were found to be predictive of cell death measured at 24 hrs. (2) Screening of the DIVERSet collection for agents that protect against cell death due to toxic levels of calcium identified twelve compounds, three of which provided significant protection against calcium-induced loss in respiratory capacity. (3) Two agents provided protection against photoreceptor degeneration in the rd1 mouse retina, similar in magnitude to the calpain inhibitor calpeptin.
Conclusions:
Defects in mitochondrial ATP production underlie a number of retinal pathologies, a phenotype that was reproduced by calcium and oxidative stress in 661W cells. These stressors diminish mitochondrial capacity in 661W cells, as seen very early in pathogenesis. On the contrary, compounds that improved mitochondrial capacity were found to provide protection for isolated photoreceptors (661W) and those present within the complex retinal network (rd1). Thus, perturbation in energy metabolism is likely to serve as an early biomarker in degeneration; and agents that ameliorate the dysregulation of energy metabolism could be developed into therapeutic strategies for treatment of retinal dystrophies.

Systemic AAV-mediated delivery of proinsulin delays visual loss in a retinitis pigmentosa mouse model

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Retinitis pigmentosa (RP) is a heterogeneous group of inherited retinal dystrophies that lead to blindness and for which there is no effective therapy. Apoptosis is a feature common to all cases of RP both in humans and in animal models. We have previously demonstrated that transgenic constitutive expression of the antiapoptotic molecule proinsulin delays retinal degeneration in the rd10 mouse model of RP. The objective of this study is to test the therapeutic potential of an AAV vector expressing proinsulin in the rd10 mouse. An adeno-associated viral 2/1 (AAV2/1) pseudotype vector expressing human proinsulin (hPi) under the control of the cytomegalovirus (CMV) promoter has been generated. At postnatal day 10 (P10), rd10 mice received a single intramuscular injection of AAV2/1-hPi or AAV2/1-null (no hPi) vector. In these mice, levels of hPi in serum and in retinal extracts were measured by ELISA, and visual function was evaluated by ERG at P30, P40, P50 and P60. Proinsulin expression was detected in serum and in retinal extracts from rd10 mice injected AAV2/1-hPi vector into the muscle as measured by ELISA. At P30, Rd10 mice injected with AAV2/1-hPi vector displaying serum proinsulin levels across three orders of magnitude demonstrated better vision than controls. Electroretinogram recording along time revealed preservation of visual function that persisted several weeks in rd10 mice injected with AAV2/1-hPi vector compared to rd10 mice receiving AAV2/1-null vector. These results open the possibility of using AAV vectors with tropism for photoreceptors or retinal pigmented epithelium to locally deliver proinsulin. Also, our observation that mice displaying serum proinsulin levels across three orders of magnitude show vision improvement suggests a broad therapeutic window for proinsulin.

The mammalian beta Heavy spectrin, an unconventional spectrin defies convention in the auditory and photoreceptor cells

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Prompted by a yeast two-hybrid screen, we found and characterized a direct interaction between the C-terminal FERM domain of myosin VIIa and spectrin beta H, the mammalian ortholog of Drosophila beta Heavy spectrin. It is noteworthy that defects in myosin VIIa cause Usher syndrome type IB, characterized by deaf-blindness in humans. We therefore analyse to role of this spectrin in the inner ear and the eye, the two sensory organs affected in myosin VIIa-defective patients. Within the mammalian auditory organ, outer hair cells (OHCs) amplify sounds by electromotility – the voltage-dependent contraction and elongation of the lateral plasma membrane (LPM). Electromotility is driven by the OHC transmembrane protein prestin, and the actin- and spectrin-based cortical lattice that underlies the LPM is also important in this process; however, the precise composition of the cortical lattice, and the way in which it interacts with prestin (defective in a human deafness), have been unknown. We found that spectrin beta H (which exists as an alpha II-beta H heterodimer that is almost twice the length of conventional beta spectrins) form the spectrin-based cytoskeleton that provides OHCs with the flexibility required for sound amplification. Beyond OHCs, we found that spectrin beta H is detected at the photoreceptor inner and outer segment junction. Based on our ongoing results, we believe that a multiprotein complex involved in an actin-spectrin cytoskeleton reorganization might take place in both hair and photoreceptors cells. In the retina, the two proteins codistribute in the photoreceptor cells. Coimmunopreicipitation experiments using rat retinal extracts led us show that spectrin beta H form a complex in vivo with myosin VIIa. In addition, we found that spectrin beta H associates with rhodopsin and also with several subunits of the microtubule-based motor proteins, kinesin II and the dynein-dynactin complex. The association of spectrin beta H with either rhodopsin or kinesin could be detected only in the maturating photoreceptors, from postnatal day 10 onwards. Altogether, our data led us suggest that beta H form a spectrin cargo adapter that bridges components of the phototransduction machinery to the actin- and microtubule-based motors, thereby contributing to their transport up to the photoreceptor nascent outer disks.

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Synaptic activity and stimulation of Type II cochlear afferents

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The afferent innervation of the cochlea consists of two types of neurons, Type I and Type II spiral ganglion neurons. Type I neurons comprise 95% of the afferent neuron population, and project to single inner hair cells (IHC) in the organ of Corti. Acoustic information of sound timing and intensity is encoded at this IHC to Type I synapse, while frequency is encoded by IHC position along the tonotopic cochlear axis. Type II afferents comprise the remaining 5% of spiral ganglion neurons. They project past IHCs to contact several outer hair cells (OHC). Type II afferent projections are thin and un-myelinated, and little is known about their synaptic inputs or excitability due to difficulties recording from the neurons. We used a novel approach of performing giga-ohm seal, whole-cell electrophysiological recordings from dendrites of Type II afferents directly under OHCs in the apical turn of rat cochleas. In pre-hearing (P5-9) rats, large tetrodotoxin sensitive sodium currents and slowly accommodating action potentials were found. Excitatory post-synaptic currents (EPSCs) were recorded that reversed polarity near 0 mV and were sensitive to NBQX, an AMPA type glutamate receptor blocker. This indicates that synaptic inputs to Type II cochlear afferents are glutamatergic and mediated by post-synaptic AMPA receptors. Type II afferents were strongly stimulated by ATP, which depolarizes and induces neurotransmitter release from OHCs, resulting in EPSCs in post-synaptic Type II dendrites. Additionally, ATP directly induced a large inward current in Type II dendrites. This effect of ATP depolarized Type II neurons and could initiate action potentials. Stimulation of Type II afferents by ATP was down-regulated, but not eliminated, after the onset of hearing in rats. Further, EPSCs were still observed in hearing (P17-19) rats.

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The dynein motor is the basis of active oscillations of mosquito antennae

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The antennae of mosquitoes are acoustical detectors, responding to sound in the form of particle displacement. Their antennae also vibrate spontaneously in the absence of stimulation. The sensory cells at the base of the antennae, which transduce the mechanical displacement into electrical impulses, are thought to underlie these spontaneous oscillations. Motility and mechanosensitivity of cilia, in sensory cells, have been hypothesised to be a complementary feature of mechanosensory transduction. Many cilia of sensory cells, which transduce sound energy, appear to be highly motile. The ability to generate mechanical forces in these cells, in the absence of stimulation, appears to be a pre-requisite for highly sensitive hearing systems.
Motility of cilia increases the sensitivity of mechanotransduction by reducing the inertia needed to push the system past threshold. Spontaneous oscillations confer frequency selectivity and possible amplification of sound induced stimuli. The oscillations themselves are hypothesised to be a feature of the mechanotransduction channel. An alternative hypothesis is that dynein, which resides along the length of the cilia, is responsible for the oscillations.
Dynein has been shown to be an oscillating force generator and resides between the microtubule filaments. By sliding the microtubule doublets relative to one another it is able to induce ciliary bending; the same ancestral mechanism that powers motile flagella found in single-celled organisms.
From mechanical and electrical measurements of the oscillations of the Johnston's organ (JO) of the mosquito (Culex quinqifaciatus) in response to temperature changes, we provide evidence to support the role of dynein in generating the oscillations. Colchicine, which disrupts polymerisation of microtubules, also blocked active oscillations. We recorded sound evoked electrical potentials from the JO, after colchicine injection, and loss of all oscillations. Transduction was not affected after blocking active oscillations. The transduction apparatus is, therefore, not responsible for these oscillations.

LiGluR-mediated visual responses in the *rd1* mouse model of retinitis pigmentosa

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Retinitis pigmentosa refers to a heterogeneous group of inherited diseases in which genetic mutations result in progressive photoreceptor degeneration. Without photoreceptor activity, visual processing cannot take place, despite the fact that second and third order retinal neurons remain functional. These diseases currently cause severe visual deficits in one of every 3,000 individuals. Currently, there are no approved therapies to slow the progression of this disease, or stop photoreceptor cell death. An alternative therapy is to restore light responsiveness to a retina devoid of photoreceptors by expressing light-activated channels in surviving neurons. We used intravitreal injection of adeno-associated virus serotype 2 (AAV2) to deliver the engineered light activated glutamate receptor, LiGluR, to retinal ganglion cells (RGCs) in the *rd1* mouse model of blinding retinal degeneration, which lacks photoreceptors at the late stages of the disease.
Single-unit recordings from retinal wholemounts show that LiGluR successfully imparts light sensitivity onto RGCs, allowing for precise and reversible lightmediated control of spiking activity. Local field recordings in anesthetized mice were used to characterize LiGluR-mediated visual responses at the cortical level in response to brief (50ms) and long (300ms) pulses of full-field illumination. Visual responses were maximal at ~380nm (corresponding to the peak wavelength sensitivity of LiGluR), and could be obtained even 48 hours postinjection of the photoswitch. Peak LiGluR-mediated cortical field potentials in response to a 300ms full-field flash (-371.5 ± 36 μV, n=13) were significantly larger ($p<0.0001$, Wilcoxon sign rank test) than those mediated by Channelrhodopsin2 (-61.7 ± 12.3 μV,n=6). These results suggest that LiGluR is a promising candidate for therapeutics aimed at restoring visual function to patients in late stage retinal degeneration.

Posters

1

Oxidative stress, proteinases / inhibitors imbalance in degenerating retinas

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The rd1 (retinal degeneration) mouse retina shows degeneration homologous to a form of Retinitis Pigmentosa with a rapid loss of rod photoreceptors and deficiency of retinal blood vessels. Due to Pde6brd1 gene mutation, β subunit of phosphodiesterase (PDE) of rd1 retina has an inactive PDE which elevates cGMP and Ca2+ ions level. In vitro retinal explants provide a system close to the in vivo situation, so both approaches were used to compare the status of oxidative stress, transforming growth factor-β1 (TGF- β1), sialylation, galactosylation of proteoglycans, and different proteinases-endogenous inhibitors systems participating in extracellular matrix (ECM) remodeling/degeneration and programmed cell death (PCD)/apoptosis in wt and rd1 mouse retinas.
Proteins and desialylated sulfated glucosaminoglycan parts of proteoglycans in ECM of rd1 retina were, respectively, decreased and increased due to enhanced activities of proteinases. Desialylation increases the susceptibility of cells to phoagocytosis / apoptosis, decreased neurogenesis and faulty guidance cues for synaptogenesis. In vivo activities of total proteinases, matrix metalloproteinase-9 (MMP-9) and cathepsin B were increased in rd1 retina on postnatal day 14 (PN14), -21 and -28, due to relatively lower levels of tissue inhibitor of MMPs (TIMP-1) and cystatin C, respectively. This corresponded with increased in vitro secretion of these proteinases by rd1 retina. Cells including end-feet of Mueller cells in degenerating rd1 retina showed intense immunolabeling for MMP-9, MMP-2/TIMP-1, TIMP-2 and cathepsin B/cystatin C, and proteinases pool was increased by Mueller cells. Intense immunolabeling of ganglion cell (RGC) layer for cathepsin B and of inner-plexiform layer of both PN2/PN7 rd1 and wt retinas indicated importance of cathepsin B in synaptogenesis and PCD of RGC.
Increased levels of TGF- β1 in vitro transiently increased the secretion of MMPs and cathepsins activities by wt explants which activate TGF- β1 and remodel the ECM for angiogenesis and ontogenetic PCD. Whereas, lower level of TGF- β 1 and persistently higher activities of MMPs and cathepsins in rd1 retinas and conditioned medium, suggested that proteinases degraded TGF-β1 and ECM and caused retinal degeneration.
Lower activities of glutathione-S-transferase and glutathione-peroxidase in rd1 retina contribute to oxidative stress which damages membranes and increased the expression, release/secretion of proteinases relative to their endogenous inhibitors. Participation of oxidative stress in rd1 retinal degeneration was further confirmed from the partial protection of rd1 photoreceptors by in vitro and/or in vivo supplementation with glutathione-S-transferase or a combination of antioxidants namely lutein, zeaxanthin, α-lipoic acid and reduced-L-glutathione. Treatment with combination(s) of broad spectrum proteinase inhibitor(s) and antioxidants needs investigation.

2

Her9 is a repressor of neurogenic fate in the zebrafish inner ear acting downstream of Tbx1

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Hairy and Enhancer of Split genes (Hes in mammals and Her in zebrafish) are bHLH proteins that act as repressors of proneural activity. Some genes of this family of proteins are expressed upon Notch pathway activation and prevent neuronal progenitors to undergo differentiation. A second class of HES genes, are expressed at high levels in non-neurogenic territories and have been described to maintain neural stem-cell properties and prevent neurogenesis in boundary domains. We have studied the role of Her9, a zebrafish ortholog of mammalian Hes1, during inner ear development. Previously we have shown that the inner ear is early patterned into a neurogenic territory and a domain devoid of neurogenic potential and we hypothesized that Her9 gene was probably a repressor of proneural activity in the non-neurogenic domain. Here, we present novel data that suggest that Her9 is one of the downstream targets of Tbx1 that mediate Tbx1-repression of neurogenesis in the non-neurogenic territory. Both Tbx1 and Her9 are established by RA in the same domain complementary to neurogenin1, suggesting a possible shared pathway. Moreover, in Tbx1 mutants (vgo) a clear inhibition of Her9 expression is detected. Finally, injection of Her9-MO leads to an expansion of neurogenesis in the inner ear but no effects on sensory macula development is observed. We are currently testing if Her9-expressing cells are slow-diving progenitors that are maintained as non-neurogenic progenitors. Taken together, our data indicates that: i) Tbx1 and Her9 are regulated by Retinoic acid, ii) Her9 is a downstream target of Tbx1, iii) Her9 is essential for maintaining a domain devoid of neurogenesis.

3

Identification of two novel mutations in the USH1C gene in Uher type 1 families from the European-Mediterranean population

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Intoduction:
Usher syndrome Type I (USH1) is an autosomal recessive disorder characterized by severe-profound sensorineural hearing loss, Retinitis Pigmentosa and vestibular areflexia. To date, five USH1 genes have been identified. One of these genes is USH1C, that was shown to encode harmonin, a PDZ domain-containing protein.
Aims:
The aim of the present work was the mutation screening of the USH1C gene in our cohort of USH1 patients in order to identify the genetic cause of the disease.
Patients and methods:
35 unrelated USH1 patients were screened for mutations in the USH1C gene by direct sequencing.
These patients had already been screened for mutations in the remaining USH1 known genes (MYO7A, CDH23, PCDH15 and USH1G), but no mutation was found.
Results:
Two novel mutations were homozygously found in two unrelated patients: a frameshift mutation (c.369delA) and a nonsense mutation (p.C224X).
Conclusion:
Our results confirm a minor involvement of the USH1C gene in the pathogenesis of Usher syndrome type 1.

4

Prevention of hair cell death in Pou4f3 transcription factor knockout mice using an anti-apoptotic factor

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A major challenge in the inner ear research field is to restore hearing loss of non-genetic and genetic origin. A large effort is being made to protect hair cells from cell death after exposure to noise or drugs that can cause hearing loss. Our research is focused on protecting hair cells from cell death occurring in a genetic model for human deafness. Pou4f3 is a transcription factor associated with human hearing impairment. Pou4f3 mutant mice have no cochlear hair cells, resulting in complete deafness. Although the hair cells appear to form properly, they progressively degenerate via apoptosis. In order to rescue the hair cells in the mutant mice, we produced explant cultures from mouse cochleae at an early embryonic stage and treated the cells with z-VAD-fmk, a general caspase inhibitor. Hair cell numbers in the mutant mice treated with z-VAD-fmk were significantly higher than in the untreated mice. We found that the time window that z-VAD-fmk have a protective effect is between E14.5 to E16.5, but not after E18.5. The protective hair cells rapidly took up the styryl dye AM1-43, suggesting that they are functional. The source of the surviving hair cells is not due to proliferation, as measured by 5-bromo-2-deoxyuridine (BrdU) labeling or to supporting cell the survival appears to be a result of the effect of the anti-apoptotic agent on the dying hair cells. By restoring genetic-based hearing loss in explants derived from mice, we are moving a step forward to restoring hair cells in individuals with hearing impairment.

5

Clinical and genetic stratified study in 283 Spanish families affected by retinitis pigmentosa using the arRP genotyping microarray

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Introduction:

Retinitis pigmentosa (RP) is a genetically heterogeneous disorder characterized by progressive loss of vision. We propose a clinical and molecular classification in Spanish families using a specific genotyping microarray.

Patients and methods:

283 unrelated Spanish families, 91 of which being diagnosed as early onset Autosomal Recessive Retinitis Pigmentosa (onset <10 years of age) and 192 as non early-onset ARRP (onset >10 years of age). An ophthalmological examination was performed in each patient. The families were also classified according to their genetic condition: 109 autosomal recessive (arRP) cases and 174 sporadic (sRP) cases (considering sRP plus consanguinity as sRP). All of them were analysed with a genotyping microarray, specific for arRP which tested 501 disease-associated sequence variants in 16 arRP genes, followed by a family study. Direct sequencing was used to confirm the results obtained by the genotyping microarray, except for one family, because of the bad quality of the sample.

Results:

At least one mutated allele was found in 19 out of 91 (21%) early onset RP and in 32 out of 192 (17%) non-early onset RP families. There were no significant differences ($p>0.05$) in the number of mutated families or in the mutated alleles between both groups. RDH12 was the gene most frequently mutated in early onset RP families (3.6% of the studied families). However, for the non-early onset RP families, the gene most frequently mutated was USH2A followed by CERKL (7.8% and 4.6% of the studied families respectively). Two false positives were detected in the early onset RP group. There were no significant differences ($p>0.05$) when the results were compared between arRP and sRP families. The sex ratio among affected individuals in the sRP families was similar (as expected) to that of arRP.

Conclusion:

The use of the arRP genotyping microarray is the first step in molecular diagnosis in Spanish families with Autosomal Recessive Retinitis Pigmentosa. The causal mutation was determined in 18% and 17% of the early onset and non-early onset RP families respectively, using the arRP genotyping microarray. RDH12 was the main gene responsible in early onset RP and USH2A in non-early onset RP families. Our results support that the sporadic cases, which account for 40-50% of the non-syndromic RP cases in Spanish population, present an autosomal recessive inheritance pattern.

6

Mutation screening of 111 Spanish families with autosomal dominant retinitis pigmentosa by genotyping microarray

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Introduction:

Autosomal dominant Retinitis Pigmentosa (adRD) is a group of clinically and genetically heterogeneous diseases characterized by progressive loss of vision. Today, more than 37 genes have been described associated with adRP but they can only explain 50% of families, making their genetic diagnosis and prognosis more difficult and expensive.

The aim of this study was to validate a specific genotyping microarray for its application to the molecular diagnosis of Spanish patients with adRP.

Patients and methods:

We analysed 111 unrelated Spanish families for whom a clinical diagnosis of adRP was suggested based on their ophthalmological examination and pedigree data.

In a first step, samples were studied by using a genotyping microarray (ADRP, Asper Biotech). Sequencing analysis followed by family study was performed in order to validate all variants detected with the microarray.

In a second step, RHO mutation screening by sequencing analysis was performed in negative samples.

Results and conclusion:

The ADRP genotyping microarray detected the mutation associated with the disease in 35 of the 111 adRP families (31.5 %) and allowed us to diagnose 149 patients. In all cases, sequencing analysis confirmed the mutations previously detected using the genotyping microarray.

As in other previously reported populations, RHO was found to be the most frequently mutated gene in adRP Spanish families (13.5%). In our cohort of patients, 27 different mutations have been detected and only 5 of them have been detected in more than one family (p.Pro347Leu and p.Asp190Tyr in the Rho gene (in 3 and 2 families respectively), p.Arg224Pro in IMPDH1 gene (in 2 families), p.Gly56Arg in NR2E3 gene (in 3families) and p.Pro51Leu in NRL gene (in 3 families).

All mutations found were confirmed by automatic sequencing. Thus, giving a high level of analytical specificity and a 3.5% of clinical sensitivity. For this reason, we consider that the use of the ADRP genotyping microarray is a quick low cost first step in molecular diagnosis of Spanish patients with adRP.

7

Systemic delivery of self complementary AAV2/9 vectors in adult mice results in efficient transduction of the retina

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Gene delivery to retinal cells using vectors derived from adeno-associated virus (AAV) usually implies sub-retinal or intravitreal injections. These procedures are relatively efficient to direct transgene expression into photoreceptors or retinal pigment epithelium (RPE) cells but their invasiveness could represent a risk for the patient. In particular, sub-retinal injections of viral vectors cause a detachment of the retina at the site of injection and can lead to subsequent localized trauma inducing retinal thinning and apoptosis of retinal cells. Moreover, sub-retinal vector injections direct transgene expression only in the injected areas of the treated eye (delimited by the "retinal bleb detachment" induced by the injection in the sub-retinal space).
We will show a new non-invasive gene transfer method that allows a substantial transduction of the retina (in both eyes) after systemic delivery of AAV vectors. Single strand (ss) or self-complementary (sc) AAV vectors of serotype 1 and 9 encoding the green fluorescent protein (GFP) or the murine secreted alkaline phosphatase (mSEAP) were intravenously injected in adult mice and transgene expression was analyzed in the retina. We found that intravenous injection of the scAAV vectors, in particular of serotype 9, led to a significant transgene expression in different cell layers of both eyes, including the choroid and the retina. This was first observed at the DNA and protein level following intravenous injection of scAAV9-mSEAP vectors. The superiority of scAAV9 vectors was then confirmed by histological analysis after intravenous injection of scAAV9-GFP and immunodetection of the transgene product. Importantly, we detected transgene expression in retinal pigment epithelium cells, photoreceptor cells, cells of the inner nuclear layer, Müller cells and cells of the ganglion cell layer (RGC), although these different retinal cell types were found to be transduced with variable efficiencies. Notably, the RGC layer appeared to be transduced with the higher efficiency. In addition, we found high level of GFP expression outside of the retina in the choroid, the ciliary body and the optic nerve.
This study shows, for the first time, that it is possible to achieve gene delivery to the retina of adult mammals after a single intravenous administration of scAAV2/9 vectors. This less invasive approach is promising for the development of gene therapy strategies for eye disorders, and especially for neurodegenerative diseases of the retina.

8
MYO7A mutations: genotype–phenotype correlation

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Mutations in the human unconventional Myosin VIIa (MYO7A) are reported to be responsible for syndromic deafness (Usher I and atypical Usher syndrome). However, mutations causing recessive (DFNB2) and dominant (DFNA11) non-syndromic deafness have also been reported. In our study, deafness segregating as a recessive trait in two unrelated consanguineous Tunisian families showed linkage to markers in the region of the MYO7A on chromosome 11q13.5. Sequencing of MYO7A gene revealed two mutations (c.470 2G>A and c.1935 G>A). These mutations were previously reported in two other Tunisian families. Patients with the c.470 2G>A are considered as suffering from Usher I on the basis of profound and congenital hearing loss, vestibular areflexia and retinitis pigmentosa. While the two families, with the c.1935 G>A mutation, present phenotypic variability ranging from non-syndromic deafness to Usher syndrome. This second mutation occurs in the last nucleotide of exon 16, encoding part of the motor domain, and substitutes a methionine by an isoleucine (p.M645I). Both methionine and isoleucine have a similar charge. In addition molecular modeling doesn't predict any major effects for this mutation. In contrast, this mutation is expected to affect the splicing efficiency. To correlate clinical phenotype variability with the molecular phenotype, we have investigated these effect changes for a possible impact on mRNA splicing. Using a minigene assay we found that the adjacent donor splice site consensus is completely abolished resulting in aberrant splicing in the case of the c.470 2G>A mutation, whereas in case of the c.1935 G>A mutation the adjacent donor splice site consensus is partially abolished. So we found two transcripts, one include the exon 16 while the other doesn't include this exon. In order to confirm this result in vivo, the effect of splice site mutation (c.1935 G>A) was assessed by RT-PCR analysis with lymphoblastoid cell total RNAs generated from affected individuals. We founded the same result. This finding could explain why the c.1935 G>A causes a less severe phenotype than the c.470 2G>A USH1B-associated allele. To explain intrafamilial phenotypic variability, we will use time reverse transcription polymerase chain reaction assays to quantitative and to compare transcription levels of mRNAs in different patients with the c.1935 G>A.

9

Endocochlear potential depends on Cl- channels: mechanism underlying deafness in Bartter syndrome IV

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Human Bartter syndrome IV is an autosomal recessive disorder characterized by congenital deafness and severe renal salt and fluid loss. It is caused by mutations in BSND, which encodes barttin, a beta-subunit of ClC-Ka and ClC-Kb chloride channels. Inner-ear-specific disruption of the Bsnd gene in mice now reveals that the positive potential, but not the high potassium concentration, of the scala media depends on the presence of these channels in the epithelium of the stria vascularis. The reduced driving force for K+-entry through mechanosensitive channels into sensory hair cells entails a profound congenital hearing loss and subtle vestibular symptoms. Although retaining all cell types and intact tight junctions, the thickness of the stria is reduced early on. Cochlear outer hair cells degenerate over several months. A collapse of endolymphatic space was seen when mice had additionally renal salt and fluid loss due to partial barttin deletion in the kidney. Bsnd-/- mice thus demonstrate a novel function of Cl- channels in generating the endocochlear potential and reveal the mechanism leading to deafness in human Bartter syndrome IV.

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Blood vessel complexity and cones viability in a mutant mouse model of retinal degeneration

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Retinitis Pigmentosa (RP) comprises a family of disorders in which photoreceptors die progressively, typically for a mutation in a rod-specific gene. Rod death is followed by secondary degeneration of cones and loss of useful sight. In human RP and in animal models of this disease, photoreceptor death is accompanied and followed by a major reduction in retinal blood vessels.
In Our work we investigate the early and late effects of photoreceptor degeneration upon cones and retinal blood vessels in the rd10 mutant mouse, a model of autosomal recessive RP.
To evaluate the blood vessel complexity and cone survival we analyze eyes from homozygous rd10 and wt control mice at different age. At least 3 retinas, from different animals, for each age group, were examined after specific stained to visualize blood vessels. Retinal samples were examined as whole mount preparations with TCS-NT or confocal microscopes. For each retinal sample, 8 fields, regularly spaced in the 4 quadrants (Dorsal, Ventral, Nasal and Temporal) and covering separately central and peripheral areas, were chosen for morphometric evaluation of blood vessels. The three blood vessel plexa were scanned at the confocal microscope along the z axis. Projection images of the three separate plexa were transferred to a Metamorph analyzer and processed to estimate the complexity of each plexus by counting the number of intersections between blood vessels and an overlying squared grid, 400 μm wide.
To marker the outer segment of cones we used a rabbit anti-Opsin revealed with Alexa-568 conjugated secondary antibodies. Cone outer segments lay in a single plane, so that retinal whole mounts result into high resolution topographic maps of cone distribution in which bright, cone-rich areas alternate with dark, cone-degenerating zones. Each retina is systematically imaged with a cooled Axiocam colour camera interfaced with a Axioscope fluorescent microscope using a 5x objective. Using differences in brightness displayed on the images as colour gradients, cones isodensity curves are then traced digitally. Cone densities within each curve are estimated separately by counting cones on high resolution confocal images. Cones are counted with the object recognition tool of Metamorph. We can assign each estimated cone density to the corresponding isodensity area on a retinal map using a contour plot graph.
We recently devised and validated this method by using the normal mouse retina. The advantage of such a systematic analysis is that it takes into account the high degree of spatial non-homogeneity in photoreceptor degeneration and scans the whole retinal surface with precise reference points.

11

Discovery of a harmonin mutation for Usher syndrome in children diagnosed with non-syndromic hearing loss

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The identification of the molecular basis of deafness in the last decade has made a remarkable impact on genetic counseling and diagnostics for the hearing impaired population. Since the discovery of the most prevalent form of deafness associated with mutations in the *GJB2* (connexin 26) gene, many other genes have been found worldwide, with a subset of these, including unique mutations, in Israel. These include *GJB2*, *GJB6*, *MYO3A*, *POU4F3, SLC26A4* and *CDH23.* One gene for otosclerosis *(OTSC4)* was mapped to a new chromosomal location, 16q21-23.2.

Usher syndrome, divided into 3 clinical subtypes, USH1-3, is a frequent cause of the combination of deafness and blindness (retinitis pigmentosa-RP). Five genes, *MYO7A*, *CDH23*, harmonin, *SANS* and *PCDH15,* have been cloned and are known to underlie different forms of USH1. USH1 is the most common and most severe, characterized by constant vestibular dysfunction, congenital profound deafness and onset of RP in the beginning of the second decade of life. Therefore, USH1 is frequently misdiagnosed as NSHL. Previously, a mutation in the R245X mutation was detected in the *PCDH15* gene in the Ashkenazi Jewish population. Our new finding is the discovery of the c.238-239InsC mutation in the harmonin gene in this same population. In both cases the mutations were identified in young children before the age of onset of the RP. Upon the detection of the mutations, the children underwent fundoscopy and ERG, showing first signs of RP. The earlier the diagnosis is made, the better chance these children have for early habilitation and being able to communicate optimally in society, even after they have lost a portion or all of their vision.

We present a diagnostic algorithm for Jewish individuals presenting with HL, depending on the mode of inheritance, ethnic origin and clinical/audiological manifestation, and taking into account the most cost-efficient method to address the molecular diagnoses available today

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Expression in the retina of proteins with a protective role against neuronal stresses: parkin, UCH-L1 and DJ-1

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The ubiquitin-proteasome system (UPS) is highly active in neurons and its dysfunction is involved in a number of neurodegenerative disorders, such as Parkinson, Alzheimer and Huntington diseases, and retinitis pigmentosa. Aberrant proteolytic degradation and oxidative damage arising from mitochondrial dysfunction are involved in neurodegeneration, both processes being interrelated. In this context, many proteins exist in the brain that are components of the UPS and/or have a role in mitochondrial homeostasis, thus working on neuronal protection against a spectrum of cellular stresses. Among these proteins are parkin (encoded by the PARK2 gene), an E3-type Ub protein ligase; UCH-L1, a deubiquitinating enzyme; and DJ-1, a sensor for oxidative stress and ROS scavenger.

A growing body of evidence exists concerning Parkinson disease-associated visual dysfunction and morphological impairments in the retina, including that from our group. However, knowledge on the UPS in the retina is scarce, and the role of its protein components in retinal aging and disease essentially unknown. In this work we set to analyze the expression of parkin, UCH-L1 and DJ-1 in the retina of several mammalian species, ranging from rodents to human.

By means of RT-PCR and immunoblotting, we found expression of PARK2, UCHL1 and DJ1 genes at the mRNA and protein levels in the neural retina and retinal pigment epithelium (RPE) of all species studied. In addition to the canonical parkin polypeptide, we also detected two smaller isoforms specific for the neural retina. In this context, extensive alternative splicing of the PARK2 mRNA was found in the retina of all mammals examined, including exon skipping and inclusion of extra exons previously undescribed. Immunoblotting analysis of UCH-L1 revealed an additional higher molecular-weight species presumably ascribable to monoubiquitinated UCH-L1, whereas a single DJ-1 isoform was detected in the mammalian retina. Immunofluorescence confocal microscopy allowed detection of the three proteins in the cell bodies and processes of virtually all retinal cell types, including photoreceptors in most cases.

Our results extend the distribution pattern of parkin and DJ-1 to the retina, where they had not been previously reported. Also, they allow to extrapolate to the retina the neuroprotective function that together with UCH-L1 they likely fulfill in the brain against a variety of neuronal stresses. Consequently, it should be of interest to screen their encoding genes for mutations or alterations in their expression levels in patients with retinal neurodegenerative diseases, such as retinitis pigmentosa.

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Increase awareness of Sjögren syndrome recognition among health care professionals - managing malpractice risks

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While dry eye tends to be a relatively common problem in many ophthalmologists' offices. Determining the causes and treatment is often still a mystery, hence dry eye is a complex disease with many underlying causes and influential factors. Consequently, there are many clinical and research tools that are used to diagnose and study dry eye. Due to the fact that many different entities can cause dry eye syndrome and the symptoms are regularly non-specific, some serious underlying clinical settings often are misinterpreted or underestimated.

About 10% of the time dry eye is the result of Sjögren's syndrome (SS), which occurs mostly in women typically in menopause and post-menopause period. In primary Sjögren's syndrome both lachrymal and salivary glands undergo lymphocytic infiltration.

SS can exist as a primary disorder or as a secondary condition in association with another well defined autoimmune process such as rheumatoid arthritis, systemic lupus erythematosus (SLE) or scleroderma. Its genetics and clinical features most closely resemble a subset of SLE patients.

Although primarily characterized by a particular form of dry eyes (keratoconjunctivitis sicca) and dry mouth (xerostomia), this condition may affect a wide variety of organ systems including skin, lung, heart, kidney, neural and hematopoietic system. SS has many features in common with SLE, but also has certain distinct types of organ involvement such as hyperglobulinemic purpura, renal involvement due to interstitial nephritis, and an increased risk of lymphoma. In this regard, primary SS patients straddle the fence of "aggressive" lymphocytes that infiltrate tissues throughout the body in addition to their infiltration of the lacrimal/salivary glands.

At the other end of the diagnostic spectrum is the patient with symptoms of dry eyes, dry mouth, a low titer positive antinuclear antibody, and vague symptoms of fatigue, myalgia and cognitive dysfunction.

Distinguishing patients with primary SS from those with fibromyalgia, and depression who have complaints of ocular and/or oral dryness (often exacerbated by medications with anti-cholinergic side effects) has been a challenge confronting those who develop classification criteria, conduct therapeutic trials, and provide clinical care.

Physicians who can understand the underlying causes of clinical conditions associated Sjögren's syndrome and the factors that may influence it, can then participate in the process of managing it. The concepts presented in this report represent a step in the direction of simplifying Sjögren's syndrome complex understanding, to arm doctors with tools that can be used to monitor this condition and evaluate the effectiveness of therapy.

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Improved retinal function in two mouse models of retinitis pigmentosa following AAV-mediated gene therapy

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Mutational heterogeneity represents one of the greatest barriers impeding the progress of gene therapies for many dominantly inherited conditions. Autosomal dominant retinitis pigmentosa caused by mutations in the rhodopsin gene (RHO-adRP) represents one such disorder, with over 100 mutations identified to date. We have proposed a strategy of gene suppression in conjunction with gene replacement as a single mutation-independent therapeutic, which in principle may represent a therapy for RHO-adRP.

In the current studies, we have explored the effects of suppression and replacement on retinal function, as assessed by ERG. In transgenic models of RP we have studied the individual aspects of the approach using adeno-associated viral (AAV) delivery. RNAi mediated suppression of RHO targeted to sites that differ in nucleotide sequence between mouse and human was evaluated using the Pro347Ser mouse which simulates human adRP. Utilising the endogenous mouse gene as the replacement we demonstrate preservation of the target cell type, the photoreceptors, and significantly improved retinal function (2-fold greater, $p<0.01$). A potential suppression and replacement therapy will require efficient suppression to be coupled with adequate RHO expression given the physiological requirement for high levels of rhodopsin in mammalian photoreceptors. Evaluation of in vivo levels of RHO from a series of AAV vectors incorporating different promoters and various elements led us to identify a construct which achieved approximately 40% of endogenous RHO mRNA expression following subretinal injection. AAV transduction of Rho-/- mouse retinas with this vector resulted in a significant rescue of the severe retinopathy present in these mice. Correctly formed rod outer segments were detected by transmission electron microscopy and significant rod photoreceptor function was detected by ERG. These studies represent significant progress towards the development of gene-based therapies for RHO-adRP.

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A high content screening platform for the measurement of trophic factors activity in rodent models of retinitis pigmentosa

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We have developed an automated cell counting platform to validate the activity of Rod-derived Cone Viability Factors (Léveillard et al., 2004, Chalmel et al., 2007) as therapeutic agents for the treatment of patients suffering from retinitis pigmentosa. RdCVF1 and RdCVF2 are encoding respectively by Nxnl1 and Nxnl2 genes. The automated cone counting platform developed for retinal explants is used both for mouse and rat models of rod-cone degeneration the rd1, rd10, rd7 mouse and the P23H rat. The specific objective was to validate this novel method by comparison to the semi-manual stereological counting as described in Mohand-Saďd et al., (1998).

Seven retinas from rd1 mouse (C3H, rd1/rd1) and their wild type isogenic controls (C3H+/+) were dissected from 15 to 90 days post-natal (PN), oriented and fixed with 4% paraformaldehyde. For each mouse, one eye was used for automated counting and the contralateral eye for the semi-manual stereological method. For both methods the cones were labelled with peanut agglutinin (PNA) coupled to Texas-Red. The novel method involves an inverted microscope linked to a computer driven motorized platform. Following automated acquisition in the depth of the tissue, cone density was measured using a specific deconvolution procedure.

The decrease in cone density following rod loss in rd1 mouse was found to be equivalent using either the semi-manual stereological method or the novel automated platform. Then, we developed a stereological automated method allowed us to reproduce exactly the data obtained previously with the non automated method.

The decrease in cone density from 35 to 100 days post-natal was also quantified in an additional rod-cone degenerative model, rd10. Regionalisation in the cone density was demonstrated for rd7 mouse model following labelling with polyclonal antibodies generated against short-wave opsin. These data allowed us to study the regional distribution of the cone loss in the rd1 mouse. We also used this platform to quantify the loss of cones in mice carrying an inactivation of the genes Nxnl1 and Nxnl2.

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Autosomal recessive retinitis pigmentosa gene analysis in Galician patients using high-throughput genotyping

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Retinitis pigmentosa (RP) is a heterogeneous group of inherited retinal dystrophies, involving photoreceptor degeneration and resulting in partial or complete blindness. To date, more than 40 loci have been implicated in RP, but known mutations only account for about 50-60% of RP cases, suggesting that more genes remain unidentified. The absence of prevalent mutation sites or major gene makes the molecular characterization of RP patients a tough task for both research and diagnostic purposes, requiring a mutational screen of all known RP genes. The recent use of novel high-throughput approaches offers the possibility to reduce time and costing of the retinal dystrophies diagnostic.

An integrated strategy based on the use of two genotyping high-throughput techniques was used to rapidly screen for mutations in causative genes in a cohort of Galician families with autosomal recessive RP (arRP). First, haplotype co-segregation analysis in family members was simultaneously assessed at 22 candidate genes of arRP and Leber congenital amaurosis (LCA) by a SNP-genotyping chip (MassARRAY, Sequenom). Candidate genes that did not cosegregate with the disease were discarded to be screened for pathogenic variants. On average, 20 and 15 genes were excluded in consanguineous and non consanguineous families, respectively. The subsequent mutational screening was done by bidirectional sequencing of coding, promoter and splicing regions of non-discarded genes. When the number of non-discarded genes remained unmanageable (> 5) after co-segregation analysis, as in smaller and lesser informative families, resequencing microarrays based on the APEX (Arrayed Primer Extension) technology were used for a faster screening of the most known mutations (about 500) in seventeen arRP genes (Asper Biotech).

Briefly, causative mutations were found in 42% of arRP families in USH2A (11%), CERKL (6%), NR2E3 (6%), PDE6A (6%), RHD12 (6%) and RPE65 (6%) genes. Among these, four mutations were previously reported and three novel variants at PDE6A, RDH12 and RPE65 genes were found in consanguineous families in homozygous form. The clinical features of patients, the segregation analysis within the families and their absence in 200 control chromosomes strongly support the pathogenicity of these novel variants.

Finally, this strategy allowed the exclusion of all 22 candidate arRP genes in about half of the analysed families. The use of the APEX genotyping microarray in these cases did not found additional variants in previously discarded genes, confirming the efficiency of the SNP genotyping co-segregation analysis. In these families we are currently performing a genome-wide search of novel RP genes.

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Towards the development of a protective gene therapy for hereditary optic neuropathies due to mitochondrial dysfunction

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Retinal dystrophies caused by mitochondrial impairment, as other mitochondrial disorders, are inaccessible to curative or palliative therapy. Visual handicaps represent along with ailments affecting vital prognosis the most feared threat for health in our societies.
Our most important goal is to provide a gene therapy mediated by rAAV2 (Adeno-Associated Virus) that can prevent Leber Hereditary Optic Neuropathy (LHON) as it is used for three clinical trials for Leber Congenital Amaurosis due to RPE65 mutations that are very encouraging for our strategy. LHON was the first maternally inherited disease associated with point mutations in mitochondrial DNA. The most common pathogenic mutations are located in ND1, ND4 or ND6 genes, encoding subunits of the respiratory chain complex I (about 95% of LHON patients). The pathology is characterized by selective death of retinal ganglion cells (RGC) and optic nerve atrophy leading to central vision loss. The course of visual impairment is generally acute or subacute, both eyes being involved sequentially. Hence, monocular vision loss provides a unique clinical situation in which it becomes possible to design and conduct clinical trials for LHON patients. Unfortunately, up to date no treatment is available for preventing vision loss in these patients. In France, it is estimated that 4000 persons suffer from LHON and there is as much as asymptomatic carriers. We have optimized the allotopic expression (optimized expression of mitochondrial genes transferred to the nucleus) for the mitochondrial genes ATP6, ND1 and ND4 and obtained a complete and sustained restoration of mitochondrial function in human fibroblasts in which these genes were mutated. Recently, we were able to obtain a heteroplasmy in retinal ganglion cells which led to the coexistence of the endogenous ND4 and the human ND4 gene bearing the G11778A mutation responsible of 70 % of LHON cases. The efficient mitochondrial import of the deleterious human ND4 protein induced the selective loss of RGCs and visual impairment. Most importantly, we were able to prevent RGC degeneration and the deleterious consequences for visual behavior by counteracting mutant ND4 expression with wild-type ND4 administrated two weeks after mutant ND4 treatment.
Our main goal is the transfer to clinic of our gene therapy protocol since undoubtedly it will represent a major step for the generation of a treatment aimed at improving life conditions of patients suffering from LHON.

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Identification of a new Usher 3 like locus

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Usher syndrome (USH) is the most common genetic disease causing both deafness and blindness. USH is divided into three groups, USH1, USH2 and USH3 depending on the age of onset, the course of the disease, and on the degree of vestibular dysfunction. By homozygosity mapping of a Dutch consanguineous family we have found a new locus for USH. The affected family members have a unique association of Retinitis Pigmentosa, progressive hearing impairment, vestibular dysfunction, and congenital cataract. The family has a phenotype close to, but not identical to previously published USH3 patients, as no report of congenital cataract has been reported for USH3. A genome-wide genotyping was performed and excluded linkage to the known 9 USH genes. By homozygosity mapping we found a new locus on chromosome 15. The locus mapped to 15q22.2-23 in a 7.4 Mb large interval.

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Otoferlin is critical for a highly sensitive calcium-dependent exocytosis at vestibular hair cell ribbon synapses

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Otoferlin, a large Ca2+ binding protein with six C2-domains, has been proposed as an essential Ca2+ sensor for neurotransmitter release at auditory hair cell ribbon synapses (Yasunaga et al., 1999; Roux et al., 2006; Beurg et al., 2008). Indeed, otoferlin knock-out (Otof -/-) mice are deaf because cochlear hair cells lack calcium-evoked exocytosis (vesicle fusion). Intriguingly, while otoferlin is also highly expressed in type I and type II hair cells of vestibular organs, Otof -/ - mice do not display apparent vestibular disorders suggesting that otoferlin might not be essential for exocytosis in all types of sensory hair cells of the inner ear.
However, we show here that the vestibular nerve compound action potentials evoked during transient linear acceleration ramps in Otof -/- mice are not normal. They display higher threshold, lower amplitude and increased latency compared to wild-type mice, suggesting a partial synaptic deficit at vestibular hair cell synapses. Using patch clamp capacitance measurement in intact vestibular utricles, we show that normal type I and type II hair cells display a remarkable linear transfer function between Ca2+ entry, flowing through voltage-activated Ca2+ channels, and exocytosis. This linear Ca2+ dependence was observed when changing the Ca2+ channel open probability or the Ca2+ flux per channel during various test potentials. In Otof -/- vestibular hair cells, exocytosis displays slower kinetics, reduced Ca2+ sensitivity and non-linear Ca2+ dependence, despite morphologically normal synapses and normal Ca2+ currents.
Overall, our data show that vestibular hair cells in contrast to cochlear hair cells still show residual exocytosis, however with changes in the calcium dependence of release and kinetics. These subtle changes in exocytosis give much better insight into the role of otoferlin in the calcium dependent process of exocytosis than the complete defect in cochlear hair cells. We conclude that otoferlin is essential for a high affinity Ca2+ sensor function that allows efficient and linear encoding of low intensity stimuli at the hair cell synapse. The residual nonlinear and poorly sensitive calcium-dependent exocytosis of Otof -/- vestibular hair cells is likely driven by other low affinity calcium sensors that remain to be uncovered.

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The activity of cGMP-dependent protein kinase (PKG) is involved in photoreceptor degeneration in two mouse models for retinitis pigmentosa

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Retinitis pigmentosa (RP) is a significant cause of hereditary blindness in the developed world and involves the degeneration of retinal photoreceptors. However, while many causative mutations have been identified, the molecular mechanisms behind RP are still not very well known. Here we have analysed the photoreceptor degeneration in a mouse model for Retinitis pigmentosa, rd1, in which the rod photoreceptors undergo an early as well as rapid cell death. The mutation in the rd1 model concerns the gene for the beta subunit of a cGMP hydrolysing enzyme in the rods, phosphodiesterase 6, and renders the enzyme non-functional, which in turn leads to cGMP accumulation in these cells. We show that the degeneration is linked to an overactivation of cGMP-dependent protein kinase (PKG). For instance, when PKG activity was induced in wild-type retinae, this was found to be both necessary and sufficient to trigger cGMP mediated photoreceptor cell death. Moreover, in an organotypic retinal explant paradigm, target-specific pharmacological inhibition of PKG activity led to a considerably reduced rd1 photoreceptor degeneration, and a similar effect was seen after PKG inhibition in vivo. Interestingly, the PKG involvement may not be restricted to the rd1 mutation since PKG inhibition was also seen to reduce the photoreceptor death in retinal explants from another model of RP, the rd2 mouse. Our results thus demonstrate an important role for PKG activity in cGMP-mediated photoreceptor degeneration and suggest PKG as a novel target for pharmacological intervention in RP.

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Wilson disease

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Background:
Wilson disease is an autosomal recessive disorder of copper metabolism.(prevalence of ~30 affected individuals per million population).
Purpose:
The purpose of this study was to determinate clinical presentation,diagnostic course and long term outcome.
Materials and methods:
We performed a retrospective analysis of 51 children with Wilson Disease,from 1998 to 2008. We used data from hospital files.
Results:
In all, 48 (94.12%) were symptomatic and 3 (5.88%) patients were presymptomatics at the time of diagnosis. 25 (49.02%) patients presented predominantly with neuropsychiatric symptoms and 23 (45.1%) with hepatic symptoms.27.45% of patients have positive family history for Wilson disease.Presenting symptoms were chronic hepatitis in 15 patients, liver cirrhosis in 18 patients, fulminant hepatic failure in 2 patients, resembling autoimmune hepatitis in 1 patients. Kayser-Fleischer rings were detected in 54.9% of patients with Wilson disease.Neuropsychiatric symptoms were motor abnormalities with Parkinsonian characteristics, dysdiadochokinesia, poor handwriting and cognitive deterioration, behaviour changes.The most frequent errors in diagnosis for the hepatic manifestation were: epidemic acute hepatitis(5), chronic hepatitis without AgHBs(3), autoimmune hepatitis(1). Concerning the neuropsychiatric manifestation, Wilson disease was misdiagnosed as myasthenic syndrome(2) and ADHD(1) and emotional disturbances(1). MRI was performed in 30 patients;7 had normal MRI aspect (among them, 3 have neurological onset); 23 have specific aspects. Out of 3 presymptomatic patients 2 of them remain presymptomatic for 2 years on treatment with D-pencillamine and 1 patient is presymptomatic for 6 years on treatment with D-pencillamine and zinc salts. 5 patients received liver transplant, 4 live and 1 died of septicemia in the immediate post-transplant period. 2 patients died of upper digestive hemorrhage and rest of the patients are having good evolution with copper chelators depending on their compliance to treatment.
Conclusion:
Patients with predominant hepatic symptoms are frequently misdiagnosed with acute/ chronic/autoimmune hepatitis. Kayser – Fleischer ring is important in diagnosing but it's not mandatory, especially in digestive onset. It is always present when neurological signs appear. Presymptomatic patients do not develop symptoms if they remain compliant to diet and treatment with copper chelators.

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Cross-modal plasticity in the classroom: multimodal language perception in school-aged CI children

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Studies of neuroplasticity in animals and humans show that sensory deprivation in one modality can strongly affect processing in other modalities (Bavelier & Neville, 2002). Recently, studies looking at congenitally deaf children who received cochlear implants (CI) early in life have shown that early auditory stimulation is crucial for (near-)typical auditory cortical development (Sharma et al., 2009). However, determining the optimal age to provide congenitally deaf children with a CI is not the only contribution of the study of cross-modal plasticity to the field of pediatric cochlear implantation. Deprivation of auditory experience increases the reliance on vision which will in turn affect the development of attention and communication skills when auditory experience is restored by cochlear implantation (Mitchell & Maslin, 2007). For example, postlingually deafened adults with a CI rely more on visual information in speech perception than controls with normal hearing and have been suggested to be better multisensory integrators (Rouger et al., 2007). However, little is known about (the development of) multisensory capabilities in children with a CI. The current study contributes to filling this gap by examining multimodal language perception in school-aged children with a CI.

More specifically, preliminary results from two experiments will be discussed. In both experiments, the performance of 10-15 congenitally children with a CI (age 6;0-8;0, all implanted before 4;0 and using their implant for more than two years) is compared to the performance of a group of age-matched children with normal hearing. The first experiment examines whether children with a CI are better multisensory integrators than their peers with normal hearing. In this experiment, children have to detect mispronunciations in quiet and noisy auditory and audiovisual listening conditions. The second experiment examines whether manual-visual information supports or interferes with audiovisual information when children with a CI have to learn new words presented audiovisually with and without simultaneous signing.

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Unraveling the role of microRNAs in the inner ear

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MicroRNAs (miRNAs) are small (17-24 nucleotide-long) non-coding RNAs processed from the transcripts of endogenous genes. Their most studied role is regulation of gene expression through the RNA interference (RNAi) pathway or inhibition of mRNA translation. MicroRNAs play an essential role in the development of the inner ear and were recently shown to be involved in deafness in both humans and mice. There are several approaches to understand the biological roles of miRNAs. One of them is to identify the exact temporal and spatial expression of microRNAs, which may indicate their possible function and help to reveal their contribution to the mechanism of hearing. For this purpose we are looking at candidate microRNA expression patterns using in situ hybridization. Another approach is to identify microRNA targets, as the microRNA-target pair is crucial for miRNA-based regulation. A single microRNA may regulate hundreds of targets; therefore, we are using bioinformatic prediction programs to predict and subsequently validate potential targets.
To achieve these goals, candidate microRNAs were chosen from expression profiling of both vestibular and cochlear sensory epithelia, and whole inner ears using microarrays printed with the mirVana miRNA Probe Set version 1 and miRCURYTM LNA microarrays. This data was then being crossed with our own transcriptomics and proteomics, as well as publically available bioinformatics, databases. In situ hybridization using Exiqon miRCURYTM LNA probes are being performed for these candidate microRNAs. Potential targets are now being validated, and the biological functions of the microRNA-target pairs are being investigated.

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Impaired vision and olfaction in the Nxnl2-/- mouse

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We have reported the bioinformatic identification of RdCVF2, a second trophic factor belonging to the Rod-derived ConeViability Factor (RdCVF) family. RdCVF and RdCVF2 are encoded respectively for Nxnl1 (Nucleoredoxin like) and Nxnl2 genes. RdCVF2 like RdCVF is secreted by rods and promote cone viability in mouse model of disease. Expression pattern of RdCVF2 is not restricted to the retina, as RdCVF, but is more broadly distributed in the sensory and central nervous system. We showed that RdCVF2 is expressed in olfactory epithelium and more precisely in olfactory neurons.
We analysed Nxnl2-/- phenotype. The visual function of the Nxnl2 -/- mice has been tested by electroretinography. The postnatal development of retinal photoreceptors in the Nxnl2-/- mice was indistinguishable from that of wt controls as judged by histology and electroretinograms (ERGs) in younger mice. However, by 10 months of age, 30% of cones were lost. Along with the loss of cones, ERG amplitudes declined compared of wt controls. This severe phenotype was restored by AAVRdCVF2 injection in Nxnl2-/- at 6 months of age.
Since RdCVF2 was detected in olfactory neurons, we analysed Nxnl2-/- olfactory function in performing some olfactory discrimination learning tests. At 2 months of age, Nxnl2-/- correct responses are similar to those of control mice. However, older Nxnl2-/- mice failed to respond correctly compared to wt mice. Then, we analysed possible viability activity of RdCVF2 on adult culture of olfactory neurons. The olfactory neurons were found to be higher when cultured in the presence of RdCVF2.
The fact that Nxnl2 encodes in addition to RdCVF2 an alternative spliced isoform, a putative enzyme RdCVF2L lead us to hypothetis a bifunctional function for RdCVF2. RdCVF2 like RdCVF is secreted by rods and promote cone viability in mouse model of disease. We showed that RdCVF2-induced cone an olfactory neurons survival is mediated through a phosphorylation of the extracellular signal-regulated kinase (ERK) 1/2. In Nxnl2-/- mouse retina, opsin seems to accumulate at the Golgi photoreceptor. Less opsin molecules are detected at apical inner segment and also in connecting cilium of the mutant photoreceptors compared to the wild type.
Our data showed that RdCVF2 and RdCVF2L could act via two signalling pathways mediating respectively survival of target cells by activation of ERK1/2 pro-survival pathways and opsin transport through the photoreceptor cilium.

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The cell line 661W as a model of photo-oxidative damage to cone photoreceptors

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Rod-derived Cone Viability Factor (RdCVF) is produced by the Nxnl1 gene that codes for a second polypeptide, RdCVFL, by alternative splicing. While the role of RdCVF in promoting cone survival was described, the implication of RdCVFL, a putative thioredoxin enzyme, in the protection of photoreceptors is presently unknown. Using a proteomic approach we have identified 90 proteins interacting with RdCVFL including the microtubule binding protein Tau. We demonstrate that the level of phosphorylation of Tau is increased in the retina of Nxnl1 -/- mice, as it is hyperphosphorylated in the brain of patients suffering from Alzheimer's disease, presumably in some cases through oxidative stress (Fridlich et al., 2009).
We have developed a cellular model of photo-oxidative damage to cone photoreceptors using the mouse derived cone cell line 661W. The cells are exposed for 30 sec. to UV light (254 nm) to a total dose of 1852 mJ/cm2. The cells are further incubated for 16 hours at 37°C (5% CO2) and the viability of the cells is quantified using the Live/dead assay on an automated cell counting platform. In order to evaluate the protective effect of the long isoform, RdCVFL, we transiently transfected the 661W cells using lipofectamine 2000. The efficiency of transfection was estimated to be more than 5%, which allowed us to measure the viability of transfected cells using fluorescent plasmid reporters of the cell counting platform. This model of photo-oxidative damage to cones will be used to study the RdCVFL signalling pathway.

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Higher susceptibility of the Nucleoredoxin-like 1 knock-out mouse to photo-oxidative damage

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Thioredoxins play a crucial role in the redox signalling of photoreceptors since these cells have an accelerated metabolism and are subjected to constant oxidative stress generated by light. In this line, light-induced damage is an attractive model for retinal dystrophies that are mediated by excessive photo-transduction signalling. Rod-derived Cone Viability Factor (RdCVF) is a novel type of growth factor identified through its ability to promote survival of cone photoreceptors. It is encoded by the nucleoredoxin-like gene Nxnl1 which belong to the family of thioredoxins. We generated Nxnl1-/- mice on a pure BALB/c background by homologous recombination and studied the retinal phenotype induced by photo-oxidative stress. Nxnl1-/- mice were exposed for 1 hour to white light ranging from 1700 to 2500 lux. The outer nuclear layer (ONL) thickness was found to be reduced when compared to control animals, 10 days after the exposure.
The difference in ONL thickness between the two genotypes was found to be higher in the inferior hemisphere of the retina. By contrast, this difference is no longer evident after illumination at 5000 lux. Electron microscopy confirms the presence of dying cells in the ONL. In order to understand the mechanism underlying this effect we quantified cell-death using two distinct DNA fragmentation assays. Interestingly, a dose response curve demonstrates that, 24 hours after exposure to 5000 lux, the Nxnl1-/- retinas show fewer apoptotic cells than the control animals. A kinetic analysis shows that for both genotypes at 5000 lux the maximum level of apoptosis is observed 24 hours after the insult. Altogether, our results are consistent with a physiological role for the products of the Nxnl1 gene, RdCVF and RdCVFL in protecting photoreceptor cells from oxidative stress. In addition, their roles in photoreceptor cell survival might be differentially regulated depending on the intensity of light.

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From mutation to mechanism: models for human hearing impairment and vestibular disorders

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Hearing loss is the most common form of sensory impairment in humans, and is often associated with vestibular defects. Many factors are known to affect hearing abilities, both genetic and environmental. More than 54 genes are known to cause deafness and vestibular abnormalities in families and vertebrate models. Today, mice are the most prevalent organism to study biological processes of human disease. In this regard, genetic and chemically altered mouse strains that display hearing impairment or vestibular dysfunction serve as an extremely important tool for the isolation and identification of novel genes that are involved in the pathogenesis of deafness, and the mechanisms underlying their function. Phenotypic analysis of different mouse strains, using various techniques, such as scanning electron microscopy (SEM), paint fill, immunohistochemistry with various antibodies, in-situ hybridization, behavioral observations and auditory testing, has and will in the future provide valuable data when assessing the pattern of expression and role of genes in the inner ear. Moreover, recent studies use mouse sensory epithelia explants for high resolution gene expression analysis, physiological experiments and even ex-vivo genetic manipulation, such as siRNA and overexpression.
In addition to the mouse, gene and protein expression is also studied in tissue cultures of relevant cell types and in the zebrafish. Despite the evolutionary distance between humans and fish, the resemblance of the zebrafish sensory patches in the inner ear and the lateral line to the mammalian vestibular and cochlear systems, respectively, and the regenerative nature of the zebrafish hair cells, make the zebrafish a valuable model. Similar to the mouse, transgenic zebrafish strains can also be generated as a model for human disease in general, and deafness specifically.
Upon the discovery of a new gene involved in the hearing mechanism, either in a human family or in a model organism, a combination of approaches, in-vitro and in-vivo, should be used in order to gain a better understanding of its' role in the inner ear and how it could be implicated in relevant pathologies.

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Synaptic exocytosis in developing chicken hair cells

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Neurotransmitter release at the hair cell ribbon synapse is well known to be stimulated by local rise of intracellular Ca2+ near voltage gated Ca2+ channels (VGCC). This Ca2+ dependent process implies an efficient coupling between the synaptic vesicle fusion machinery and the VGCC by mechanisms that are still largely unknown. Remarkably, during development, chick auditory hair cells switch progressively from being spontaneously active hair cells, firing action potentials mostly driven by T-type Ca2+ channels, to quiescent mature hair cells that mostly express L-type Ca2+ channels. The goal of the present study was to characterize the progressive changes occurring in the Ca2+ dependence of the hair cell synaptic machinery during development.
Hair cell exocytosis (vesicle fusion) was recorded in the intact chick basilar papilla from E10 to P2 by monitoring changes in membrane capacitance (ΔCm) during various voltage stimulations. Exocytosis associated with Ca2+ current activation could be recorded at all developmental stages examined, with a significant increase in ΔCm amplitude (~1.5 fold) and Ca2+ efficiency after ~E12-16. Mibefradil and nickel, two potent selective blockers of T-type VGCC, largely inhibited ΔCm and ICa in developing hair cells up to E10-E16 but not in more mature hair cells (>E18). Varying intracellular Ca2+ buffering, when using 2 mM EGTA instead of 0.5 mM EGTA, showed that the fast exocytosis process (RRP) was largely affected when rising EGTA in early developing hair cells (E10–E16) but not in mature hair cells (E18 and older). Overall, our data show that exocytosis is driven with a poor Ca2+ efficiency by T-type VGCC in early developmental spontaneous active hair cells. This can be explained by a reduced Ca2+ sensitivity of the synaptic machinery at these early stages of development or/and a loose spatial association of the T-type VGCC with the synaptic machinery (microdomains) as compared to L-type VGCC in mature hair cells (nanodomains).

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A mouse mutant exhibiting a unique pattern of cochlear inner hair cell degeneration

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Deafness is the most common sensory deficit in the human population, but the genetic basis for nonsyndromic hearing loss is still largely unknown. It is thought that hundreds of genes may be required for development and function of the ear, many of which remain undiscovered. Deaf mouse mutants are a powerful tool for discovering genes which affect hearing and characterising the pathways which, when perturbed, result in dysfunction of the ear.
Bronx waltzer is a spontaneous autosomal recessive mutant which has been previously described (Deol and Gluecksohn-Waelsch, 1979). Homozygotes demonstrate rapid degeneration of vestibular hair cells and cochlear inner hair cells at a late embryonic stage, resulting in deafness and vestibular dysfunction (Whitlon, 1996, Cheong and Steel, 2002), but outer hair cells remain intact. The mutation has been mapped to a 2.7Mb region on chromosome 5, but exon resequencing has failed to find a potential causative mutation (Bussoli et al, 1997, Taylor, 2005). We have carried out comparative genomic hybridisation using custom arrays to detect copy number variation, and microarrays on embryonic stages to compare the transcriptome of heterozygous and homozygous animals, and have found several candidate genes, including Trpv4, which is known to be expressed in hair cells. We are currently investigating these candidates, their expression and function. The pattern of degeneration in bronx waltzer is unique, so characterisation of the gene responsible for the phenotype will enhance understanding of the development of the ear and potentially lead to more effective therapies for hearing loss.

Bussoli, T.J., Kelly, A. and Steel, K.P. Localization of the bronx waltzer (bv) deafness gene to mouse chromosome 5. Mamm Genome 8, 714-717 (1997).
Cheong, M.A. and Steel, K.P. Early development and degeneration of vestibular hair cells in bronx waltzer mutant mice. Hear Res 164, 179-189 (2002).
Deol, M.S. and Gluecksohn-Waelsch, S. The role of inner hair cells in hearing. Nature 278, 250-252 (1979).
Taylor, A. Molecular Characterisation of the Candidate Region for bronx waltzer: a Mouse Model of Hearing Impairment. (2005).
Whitlon, D.S., Gabel, C. and Zhang, X. Cochlear inner hair cells exist transiently in the fetal Bronx waltzer (bv/bv) mouse. J Comp Neurol 364, 515-522 (1996).

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Evaluation of refined models of hearing loss caused by treatment with ototoxics or by excessive noise exposure in adult mice and rats

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Sensorineural hearing loss is a chronic disease, inherited or secondary to otic insults including ototoxics and excessive noise exposure. Mouse models are required to study the molecular bases of hearing loss and to evaluate new therapeutic approaches. Non-invasive techniques allow longitudinal studies of hearing on mouse cohorts; in particular the auditory brainstem responses (ABR) provide precise, detailed and quantitative information (http://www.iib.uam.es/servicios/nine/en/intro.en.html). Mouse models of noise-induced hearing loss (NIHL) provide information on the cellular and molecular correlates of noise injury, but standardization of noise conditions is essential to ensure experimental reproducibility. We have designed a reverberant chamber to obtain an exposure area with the highest sound level and new violet and violet swept sine noises, with a high-pass filtering and linear with frequency gain, to adapt the noise stimuli to rodent hearing (1). Different patterns of severity and chronicity were achieved depending on the stimulus selected. Strain selection determines the genetic influence to noise susceptibility. To explore if albino mice are more sensitive to noise insults and the potential protective actions of melanin pigments, ABR studies were performed to compare hearing of NMRI (Tyrc) albinos with that of the transgenic mice strains YRT2 (with functional tyrosinase, agouti) and TyrTH (with tyrosine hydroxilase under Tyr promoter, L-DOPA+, albino). Our results show that recovering the synthesis of melanin precursors protects albino mice against NIHL (2). Systemic administration of aminoglycosides is a traditional procedure to induce ototoxicity in animal models, but adult mice require high doses to achieve cochlear damage, doses that cause secondary effects and increased lethality. Our data indicate that systemic kanamycin administered intramuscularly caused a reproducible bilateral hearing loss with reduced secondary effects (3). Alternatively, in the rat, local administration of kanamycin and furosemide through a sponge located on the round window after surgical bullostomy induced cochleotoxicity without secondary effects and leaves the contralateral ear as intra-animal control (4)

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(1) Cobo et al., Applied Acoustics 70 (2009) 1034–1040.
(2) Murillo-Cuesta et al., Pigment Cell and Melanoma Research (2009) (Submitted).
(3) Murillo-Cuesta et al., Laboratory Animals (2009). In press.
(4) Murillo-Cuesta et al., Otolaringlogy Head and Neck Surgery (2009). In press.

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Study of the autosomal dominant deafness associated with DFNA5 gene in the Spanish population

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Autosomal dominant non-syndromic hearing impairment (ADNSHI) is a group of monogenic diseases highly heterogeneous genetically. This fact and the absence of clinical features specifically associated with the different genes involved makes difficult its study. In this work we have followed a candidate gene approach to obtain the mutational spectrum associated with DFNA5 gene in our cohort of genetically undiagnosed families with ADNSHI. DFNA5 maps to 7p15 and comprises 10 exons. All the mutations reported so far in DFNA5 are located at the intronic sequences flanking exon 8, leading in all cases to the skipping of this exon that is predicted to create a shift in the reading frame generating a stop codon at position 372, thereby resulting in a prematurely truncated DFNA5 protein. In this study we have analysed by DHPLC amplimers encompassing the exon 8 and flanking intronic sequences obtained by PCR from probands of 221 ADNSHI families analysed. This has enabled the identification of DFNA5 pathogenic mutations in three independent families. In patients of two of these families we have found the IVS7-16delCTT intronic mutation, which it had been previously identified in DFNA5 families of Chinese origin. The remaining mutation was novel and affected the acceptor splicing site of intron 7 (IVS7-2A>G). It co-segregated with the hearing loss in the family and it was not found in 150 normal-hearing controls. We have also investigated the effect of this mutation at mRNA level. As for the four previously identified deafness-causing mutations in DFNA5, the IVS7-2A>G results in the skipping of exon 8 in the mutant transcript. Our findings provide further support to the hypothesis that DFNA5-associated hearing loss is caused by a very specific gain-of-function mutation.

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Cochlear outer hair cell undergo an apical circumference remodeling at early post natal developmental stage

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Cochlear outer hair cell acquire electromotility and mechanotransduction at post-natal development stage. The apical junctional complex organize in a peculiar tight adherens junction in parallel. We analyze by immunohistochemistry and Fourier interpolation the apical circumferential shape of outer hair cell.

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Identification of cone protecting molecules from natural compounds libraries

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Since the isolation of morphine about 200 years ago, plants are considerate as an excellent source of secondary metabolites with biological activities. Plant secondary metabolites include a vast array of compounds that currently represent more than 200,000 defined structures. The immense chemical diversity of plants secondary metabolites is an essential part of the strategies of plants to cope with the adversities of hostile environment (e.g. protection against animals and pathogens,…). Each plant population processes its unique set of compounds well adapted to particular demands of the plant's ecological niche. The fact that these plant secondary metabolites contain molecules with pharmaceutical activities was empirical discovered very long time ago since the first fossil record of human use of plants as medicines dates from the Paleolithic, more than 60,000 years ago. Plants were used extensively by traditional herbal medicine. Several milestones in the history of drug therapy have been discovered from ethnomedical knowledge. A recent analysis of natural products as sources of new drugs indicates that over 60% of the new chemical entities can be related to natural products one way or another. At the beginning of the nineteenth century, a key step towards modern drug discovery was made when it became possible to isolate the pharmacologically active substances that are responsible for the observed effects.
Retinitis Pigmentosa (RP) and other inherited retinal degenerative diseases affecting up to 1,500,000 people worldwide. The first clinical signs are night blindness followed by the secondary loss of central vision due to the secondary degeneration of cone photoreceptors. Medically, the loss of cones is causing the biggest visual handicap because cones are essential for diurnal, colour and central vision. In order to identify the mechanism leading to secondary cone death, we have use a High Content Screening (HCS) approach to identify Rod-derived Cone Viability Factors (RdCVFs) by screening a retinal cDNA by expression cloning. Using this HCS platform, we have now obtained evidence for the existence of secondary metabolites active on cone viability in extracts from two plants used in traditional medicine to ameliorate vision. Our ongoing efforts are to isolate the active molecules from these plants and to screen a large collection of plant extracts selected for their biodiversity.

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Characterization of the Mer tyrosine kinase domains required for retinal phagocytosis

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Mer tyrosine kinase (MerTK) receptors are expressed at the apical surface of retinal pigment epithelium (RPE) cells. These receptors are required to internalize photoreceptor outer segments tips (POS) that are shed once daily. Rodent strains defective for MerTK develop retinal phenotypes resembling human retinitis pigmentosa. In humans, mutations were observed in patients with cone-rod and rod-cone dystrophies. Some patients display fluorescence in the fundus, suggesting that phagocytosis was not completely absent. Despite its obvious importance, regulation of MerTK function during RPE phagocytosis remains poorly understood. Therefore, we set out to characterize amino acids crucial for MerTK to internalize POS. We cloned rat MerTK cDNA and used site-directed mutagenesis to produce several point mutations in different intracellular domains of MerTK. When targeting the tyrosine residues, we either constitutively inactivated (Tyr to Phe) or activated (Tyr to Glu) them. We then analyzed effect of these mutations on the phagocytic capability of transfected MerTK-deficient NRK-49F and RPE cells by challenging them with FITC-labeled POS and quantifying the corresponding signals. We followed kinetics of POS binding and internalization every 30 minutes for 3 hours. As expected, absence of activity of the kinase domain impaired POS phagocytosis. Some inactivated residues showed increase of phagocytosis, suggesting a negative regulation of these particular tyrosine residues. Interestingly, 2 phases seem to emerge from these experiments, a noticeable change of phagocytic capability being detected at the 90-minute time-point. Surprisingly, most changes impacted mostly the binding characteristics even if we used mutants for the internalization receptor. In general, NRK-49F fibroblasts phagocytosed POS more slowly than RPE-J cells that are professional phagocytes. Moreover, impact of mutations was bigger on RPE-J cells than on NRK-49F fibroblasts. This might be explained by the fact that NRK-49F cells are not used to phagocytose POS and do not naturally express MerTK in contrast to RPE-J cells. Understanding how MerTK receptor's function is regulated to perform this crucial phagocytic task will give us insights into normal and pathologic retinal function, and could help us design therapies for specific forms of retinal dystrophies.

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The mammalian βHeavy spectrin in the inner ear hair cells and photoreceptor cells

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Within the mammalian auditory organ, outer hair cells (OHCs) amplify sounds by electromotility, the voltage-dependent contraction and elongation of the lateral plasma membrane (LPM). Electromotility is driven by the OHC transmembrane protein prestin in association with actin. The spectrin-based cortical lattice that underlies the LPM is also important in this process; however, the precise composition of the cortical lattice and the way in which it interacts with prestin (defective in a human deafness), have been unknown. We found that spectrin beta H (that is almost twice the length of conventional beta spectrins and exists as an alpha II-beta H heterodimer) forms the spectrin-based cytoskeleton that provides OHCs with the flexibility required for sound amplification.

In the inner ear, whereas myosin VIIa is distributed uniformly in inner hair cells and outer hair cells, βHeavy spectrin is especially abundant in the cortical lattice. The differential βHeavy spectrin distribution according to the hair cell type strongly agues in favour of an important role in electromotility.

We analysed the spectrin distribution in eye and found that spectrin beta H is detected at the photoreceptor inner and outer segment junction. Coimmunopreicipitation experiments using rat retinal extracts led us to show that spectrin beta H forms a complex in vivo with myosin VIIa. In addition, we found that spectrin beta H associates with rhodopsin and also with several subunits of the microtubule-based motor proteins, kinesin II and the dynein-dynactin complex. Altogether, these data led us to propose that a βHeavy spectrin-mediated multiprotein complex is involved in cytoskeleton reorganization that might take place in both hair and photoreceptors cells.

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The role of histone deacetylase (HDAC) activity during photoreceptor degeneration

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Inherited retinal degenerations, collectively termed Retinitis Pigmentosa (RP), constitute one of the leading causes of blindness in the developed world. RP is at present untreatable and the underlying neurodegenerative mechanisms are unknown, although the genetic causes are often established. Acetylation and deacetylation of histones, carried out by histone acetyl transferases (HATs) and histone deacetylases (HDACs), respectively, affects cellular division, differentiation, cell death and survival. Using an in situ HDAC activity assay, we found that overactivation of HDAC classes I and II accompanied photoreceptor degeneration in the rd1 human homologous mouse model for RP. This corresponded to decreased protein acetylation in degenerating photoreceptors and was co-localized with markers for cell death. Moreover, pharmacological inhibition of HDACs I/II activity in rd1 organotypic retinal explants decreased activity of poly-ADP-ribose-polymerase (PARP) and strongly reduced photoreceptor cell death. These findings highlight the importance of protein acetylation for photoreceptor cell death and survival and propose certain HDAC classes as novel targets for the pharmacological intervention in RP.

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Role of Semaphorin 6A in retinal development

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In the retina the cell bodies of each type of neurons are localized and project in specific layers. Little is known about the molecules that control the formation of these layers. How migrating neurons recognize their appropriate layer? How do they identify their target cells? We have tried to determine if Semaphorins, that are secreted or transmembrane axon guidance proteins play a role in this process. The main receptors for semaphorins are plexins. We found that semaphorin6A as well as one of its receptor, PlexinA2 are expressed in the ganglion cell layer and in the internal nuclear layer (INL) of the mouse retina. PlexinA4,the other receptor for Sema6A, was only detected in the INL. We are analyzing in details, at all development stages, the phenotype of the Sema6A and PlexinA2 mutant mice. So far, we found that in Sema6A mutant mice, several retinal layers are strongly disorganized. The internal plexiform layer (IPL) is severely disrupted, and amacrine cells are displaced. In plexinA2 mutant mice, one of the IPL sublamina is missing. In addition, the projection of dopaminergic retinal neurons is abnormal.
We are currently using different technics to analyze the function of these molecules. For instance, we are using in vitro experiments to test the role of Sema6A on the different neuronal cell types. Preliminary studies show that Sema6A does not collapse retinal ganglion cell growth cones. Next We will test Sema6A activity on bipolar and horizontal cells.

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Bclaf1, a new transcription factor involved in the differentiation of retinal progenitor cells

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Purpose:

Cell division and differentiation of retinal progenitors are under the control of endogenous and exogenous factors. In order to identify genes regulated in the developing retina, we have previously used a subtractive hybridization approach in rat retinal explants during retinal development. Our approach has revealed a candidate in the form of Bcl-2 associated transcription factor (Bclaf1), previously described as a novel death-promoting transcriptional repressor that interacts with Bcl-2-related proteins.

Methods:

Bclaf1 expression was analyzed by immunohistochemistry. Ex vivo electroporation of shRNA has been used for silencing endogenous Bclaf1 mRNA into retinal explants to examine the role of Bclaf1 during retinal development. The effect of the misexpression of Bclaf1 has been evaluated by the same approach. Retinal explants were maintained for 3 to 7 days in vitro (Div) before dissociation and counting different retinal cell types by immunocytochemistry.

Results:

Immunohistochemistry experiments revealed that Bclaf1 is expressed at different embryonic (E14, E16 and E18) and post natal stages (from P0 to P14) early in retinal progenitors cells (RPCs), and later in some differentiated amacrine, horizontal and bipolar cells from the inner nuclear layer (INL). Electroporation of retinal explants from E16 and E19 rat embryos, with a plasmid coding for a shRNA silencing Bclaf1 and eGFP reporter gene, demonstrated a decrease in the number of Pax6- and AP2-positive cells among the electroporated GFP-positive cells. After 3 Div, two fold less number of double GFP/PCNA-positive cells (progenitors) were observed in shBclaf1-electroporated explants compared to the control. At P0, down-regulation of endogenous Bclaf1, decreased the number of Pax6-positive (amacrine and ganglion cells), and enhanced the number of Rhodopsin- and Recoverin-positive cells (photoreceptors). Interestingly misexpression of Bclaf1 by electroporation of P0 retinal explants resulted in a “mirror phenotype”, with a moderate decrease in the number of photoreceptors.

Conclusions:

Our results demonstrate that Bclaf1 could be required for the early temporal competence stage in which retinal progenitors can generate amacrine and horizontal cells. Bclaf1 might participate in the development of these early differentiated retinal cells from the INL and that inhibition of its expression in retinal progenitors could promote the photoreceptor differentiation.

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Reduced Myosin VIIa expression impairs development of cochlear inner hair cell functional properties

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Mutations in the gene that encodes the unconventional, non-muscle myosin, Myosin VIIa, are associated with Usher syndrome Ib (1), a rare genetic disorder that leads to deaf-blindness in humans, as well as non-syndromic deafness (2). In mice, the orthologous recessive gene localizes to Shaker-1 also causing deafness (3) with impaired cochlear function and progressively disorganized hair cell stereocilia (4). Mechanotransduction in hair cells of mutants deficient in Myosin VIIa is impaired requiring force on the bundle beyond the physiological range (5).
We demonstrate, for the first time, that adult Myo7a(6J)/Myo7a(6J) (P20-P30) inner hair cells retain immature ionic properties. The fast, outward potassium current IK,f, normally expressed by ~P12, is absent: measured at 0 mV it was -210+/-33 pA (n=6) in Myo7a(6J)/Myo7a(6J) and 5200+/-1400 pA (n=5) in +/Myo7a(6J) controls; $p<0.01$. The cells display immature-like stimulated spiking behaviour (n=8) unlike the typically mature graded receptor potential of age-matched controls (n=4).
Surprisingly, neonatal (P2-P4) Myo7a(6J)/Myo7a(6J) mutants (n=10) show spontaneous and evoked spiking behaviour like +/Myo7a(6J) controls (n=8). The potassium currents of neonatal (P2-P4) Myo7a(6J)/Myo7a(6J) mutants and +/Myo7a(6J) appear similar: 2470+/-290 (n=9) and 2470+/- 370 (n=8) respectively measured at –25 mV.
This data explicitly shows that lack of myosin VIIa causes dysfunctional development of inner hair cell function.

1. Weil et al. (1995) Nature 374: 60-61
2. Liu et al. (1997) Nature Genetics 16: 188-190
3. Gibson et al. (1995) Nature 374: 62-64
4. Self et al. (1998) Development 125: 557-566
5. Kros et al. (2002) Nature Neuroscience 5: 41 - 47

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Identification of interactors of myosin IIIA, a protein essential for hearing

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Hearing loss is the most common sensory loss, affecting 1-2 in 1000 newborns and up to half of the population at the age of 80. The known deafness-causing genes encode a variety of proteins representing different functional families such as gap junction proteins, transcription factors, and motor proteins. Stereocilia formation, hair cell bundle organization and mechanosensory function depends on several unconventional myosins that when mutated cause stereocilia defects and deafness. Myosin IIIA is a member of this group, with a motor head domain, short regulatory neck domain, variable tail domain and unique amino terminal kinase domain. Myosin IIIA was first identified in Drosophila melanogaster, with restricted expression in the eye photoreceptor cells. In humans, myosin IIIA is expressed both in the retina and in the inner ear. Mutations in the gene encoding this molecular motor, MYO3A, lead to human hearing impairment. In a single large Israeli kindred, three different mutations in MYO3A lead to DFNB30, a progressive recessive hearing loss (Walsh et al. PNAS 2002).

To decipher the function of myosin IIIA, putative binding partners were identified using a mouse inner ear-specific yeast two-hybrid library we constructed. The library was screened with a portion of the myosin IIIa tail as bait, leading to the isolation of 48 candidate interacting proteins. Proteins were chosen for further characterization, including localization in the inner ear and co-localization with myosin IIIa. Validation of interactions was performed by Flag pull-down assays, using total cochlear extracts. A known interactor of myosin IIIa in Drosophila, Inad, whose role in the ear is unknown, was evaluated and found to be expressed in the stereocilia of mouse inner ears.

Localization of myosin IIIA, known to be in the stereocilia tip, and its interacting proteins in the organ of Corti, may direct us to better understand the protein network in which this protein is involved and its relation to auditory dysfunction.

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The homeobox gene Chx10/Vsx2 regulates RdCVF promoter activity in the inner retina

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Rod-derived Cone Viability Factor (RdCVF) is a trophic factor with therapeutic potential for the treatment of retinitis pigmentosa and other inherited retinal degenerations, a group of retinal diseases that commonly result in blindness. RdCVF protein is encoded by Nucleoredoxin-like 1 (Nxnl1), a gene that shares homology with the family of thioredoxins that are known to participate in the defense against oxidative stress. RdCVF expression is lost after rod degeneration in the first phase of retinitis pigmentosa, and this loss has been implicated in the more clinically significant secondary cone degeneration that often occurs. Here we describe a study of the Nxnl1 promoter using an approach that combines bioinformatics and transcriptomic analysis. By screening the Nxnl1 promoter with selected candidate transcription factors, chosen based upon their expression pattern, we identified the transcription factors Chx10/Vsx2, Pax4, Sp3 and Vsx1 that activate the Nxnl1 promoter. Synergistic activities were also observed between Chx10/Vsx2 and homeobox genes. In addition, Chx10/Vsx2 binds to the Nxnl1 promoter in vivo. Since Chx10/Vsx2 is expressed predominantly in the inner retina, this finding motivated us to demonstrate that RdCVF is expressed in the inner as well as the outer retina. Interestingly, the loss of rods in the rd1 mouse, a model of retinitis pigmentosa, is associated with decreased expression of RdCVF by inner retinal cells as well as by rods. Based upon these results, we propose an alternative therapeutic strategy aimed at recapitulating RdCVF expression in the inner retina, where cell loss is not significant, to prevent secondary cone death and central vision loss in patients suffering from retinitis pigmentosa.

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SLC26A4 mutation prevalence and spectrum in 114 Danish Pendred syndrome probands

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Pendred syndrome (PDS) is the most common cause of syndromic deafness, accounting for more than 5% of all autosomal-recessive hearing loss cases. It is characterized by sensorineural hearing loss and goiter and is caused by mutations in the SLC26A4 gene, encoding pendrin, a transmembrane anion transporter.

In the present study, 114 unrelated probands were sequenced in SLC26A4 and subjected to MLPA analysis for detection of deletions and duplications in cases where only one or no SLC264 mutations were identified. Novel missense mutations were analyzed for their effect on pendrin subcellular localization by immunocytochemstry .

A total of 90 of the 114 subjects (79%) were observed to have pathogenic SLC26A4 mutations. Two mutations were identified in 77 (68%) patients, one mutation in 13 (11%) patients and no mutations in 24 (21%) patients. A total of 30 different SLC26A4 mutations were identified: 18 missense mutations, 6 splice site mutations, 4 small deletions, one nonsense mutation and one multi-exon deletion. Eleven of the mutations are novel (six missense mutations, two splice site mutation, two small deletion mutations and one multi-exon deletion mutation). The mutations, p.T416P (22%), p.V138F (17%), p.L236P (12%), p.E29Q (7%), IVS8+1G>A (6%), and p.E384G (5%), were the most frequent. Interestingly, the p.V138F mutation was the second most prevalent mutation. Haplotype analysis for markers close to SLC26A4 suggests a common founder for the p.V138F mutation in our patient cohort. Finally, we demonstrate that the novel pendrin missense mutations analysed here precluded the proper localization of pendrin at the plasma membrane, demonstrating that these mutations abolish pendrin function and are disease-causing.

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Genetic dissection of hindbrain axonal commissures

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In all bilateria, a variety of axon tracts cross the midline of the central nervous system, forming well-defined commissures. In mammals, the function of commissures is well established in the forebrain and visual system, but much less understood at other axial levels. We used the emerging knowledge of axon guidance mechanisms to dissect the function of several commissures in the hindbrain. It has been shown that the receptor Robo3 acts as a master regulator of midline crossing in growing axons in mice and humans. Moreover, patients suffering from Horizontal Gaze Palsy with Progressive Scoliosis disease (HGPPS) bear homozygous Robo3 mutations, and have uncrossed dorsal median lemniscus and pyramidal tracts. We show that site-specific deletions of the Robo3 receptor using the Cre-lox system result in the absence of select commissures in the sensory, motor or sensorimotor systems and lead to specific functional deficits. Krox20::cre;Robo3lox/lox mice are lacking commissures in the 3rd and 5th rhombomeres. Although the abducens (VIth oculomotor nerve) is still projecting to the lateral rectus muscle, DiI tracing reveals the absence of internuclear crossed projection from the abducens to the oculomotor (IIIrd) nucleus. These mice exhibit defects in horizontal (but not vertical) eye movements. They also have uncrossed projection from the the aVCN (anterior Ventral Cochlear Nucleus) to the MNTB (Median Nucleus of the Trapezoid Body) associated with abnormal Acoustic BrainStem Response diagrams. Ptf1a::cre;Robo3lox/lox mice are strongly ataxic and have mostly uncrossed axonal projections from the Inferior Olive. Some Cre;Robo3 lines represent good models for HGPPS and other human syndromes. Thus, the Robo3 conditional allele is a powerful genetic tool to probe the precise functions of individual commissures and to identify the cellular substrate of related human dysfunction.

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Autophagy in otic neuroblasts is essential for acoustic-vestibular ganglion development

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Autophagy is a well characterized process at the molecular level, by which eukaryotic cells degrade parts of their own cytoplasm in response to several stress conditions. This mechanism allows cells to adapt to environmental and/or developmental changes. Autophagy has important roles in stem cell development, immune defence, programmed cell death, tumour suppression and prevention of neurodegeneration. Up to now, there are not studies exploring the participation of autophagy in inner ear development.

We will discuss the expression patterns of the key autophagy execution molecules during the early development of the chicken inner ear. In explanted cultures of otic vesicles the pharmacological inhibition of autophagy caused morphological alterations and an increase in cell death numbers, as evidenced by the accumulation of TUNEL-positive cells in the anterior part of the otic vesicle, the neurogenic zone and in the auditory-vestibular ganglia. Both insulin-like growth factor 1 (IGF-1) and substrates for the tricarboxilyc acid cycle were able to decrease the cell death caused by the inhibition of autophagy, suggesting that it has a nurturing role during inner ear development.

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HEarSpike: a biological pathways resource for the auditory system

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Biological pathways that underlie cellular processes form a tangled web of tightly regulated interlocking partners. Data on these regulatory networks is available from many sources and the merging of these data, as well as visualization and interpretation of the complexes, has become a main need in biological research. The inner ear, composed of the auditory and vestibular systems, have hundreds of proteins expressed in distinctive cell types, and understanding the pathways in which they are involved is a major challenge, which once met, will greatly enhance our ability to understand hearing.
To cope with this challenge, we are using the SPIKE tool, a knowledge-based tool of signaling pathways developed at Tel Aviv University (Elkon et al., BMC Bioinformatics, 2008). SPIKE contains three main software components: 1) A database (DB) of biological signaling pathways, collected from literature and large public sources. 2) A visualization package that allows interactive graphic representations of regulations and interactions stored in the DB. 3) An algorithmic engine that analyzes the networks for novel functional interplays between network components and merges information from different networks. SPIKE is designed and implemented as a community tool and therefore provides a user-friendly interface that allows registered users to upload data to the SPIKE DB. The DB is being inserted by multiple groups in the research community, where each group contributes data in its field of expertise. Spike combines the data inserted by all curators, making it a powerful platform for the analysis of signaling networks and the integration of knowledge.
In HEarSpike, the network was constructed on two levels, beginning arbitrarily with the myosin VIA protein, since it has known interactions. Entering this protein into Spike brought up all the interactions of myosin VIA already in in other systems. All the proteins that appeared on the map were checked in the literature for expression in the ear, which were left on the HEarSpike map, while others were hidden. On the second level, all the interactions/regulations reported in the ear were inserted into the network. Each new protein inserted brings up previously inserted interactions from other Spike networks, and the new proteins, in turn, need to be checked for expression in the ear. If expression was confirmed, the proteins were left on the map and a search of the literature was performed for their interactions and regulation in the inner ear.

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Stem cells for therapy of rare diseases. Properties, methods and challenges in life science and nanomedicine

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Developments and progress in studying of stem cells, at last years, could be accepted as a novel tool in molecular, cellular biology and medicine. It is known that stem cells could be found in most multi-cellular organisms, [1]. “The two broad types of mammalian stem cells are: embryonic stem cells that are isolated from the inner cell mass of blastocysts, and adult stem cells that are found in adult tissues”.
The aim of the work, presented could be formulated as follows:
- To analyze and to discuss the definition of a stem cell, and properties, Self-renewal (the ability to go through numerous cycles of cell division) and Potency (specifying the differentiation potential to different cell types) of stem cell, [2].
- Totipotent stem cells, Pluripotent stem cells, Multipotent stem cells, Oligopotent stem cells and Unipotent cells, have been analyzed as well, [2,3].
- Molecular, Cellular and Genetic Studies of AMD (age-related macular degeneration), Cellular Therapy for AMD, Characterization of hESC (Embryonic Stem Cells) – RPE (the retinal pigment epithelium), have been analyzed too, [4].
- Stem cells therapies for treatment of central nervous systems (CNS), Molecular Regulation of OE (the olfactory epithelium) Neurogenesis In
Vitro, have been presented as well, [1].
- Neurogenesis in the Adult Brain, Functions of Adult Neurogenesis, DNA Methylation, Growth Factor and Neurovascular Components of Stem Cell has been analyzed in [1].
- Optimized Growth of Human Embryonic Stem Cells, Adult Stem Cell therapy for Tissue Regeneration has been discussed too.
In conclusions, have been pointed out future solutions and challenges in development of stem cells therapeutic approaches.

[1] Stem Cell, Research and Therapeutics, James Lui, Springer, 2008
[2] Hans R. Scholer, Humanbiot. As Soc. Chall., Ashagate Publish, Ltd. 2007
[3] Ulloa-Montoya F., J. Biosci. Bioeng., Ltd., 2005

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A compound heterozygous mutation G to A at 380 in Connexin 26 results in profound deafness associated with a large spectrum of phenotypic modifications

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In our study, we performed mutation screening for 35delG GJB2 in non-syndromic hearing loss families, including those with cases of sporadic deafness, which were compatible with recessive inheritance. Peripheral blood lymphocyte DNA was used to amplify by polymerase chain reaction the Cx26 coding region, followed by 35delG mutation detection screening and complete sequencing.
In this case, the child was found to have profound bilateral sensorineural hearing loss. He also had moderate to severe mental retardation, motor retardation, scoliosis and kyphosis, cup ear deformity, convergent strabismus. His brother (6 years old) and sister (2 years old) were normal. Because he was adopted we can't investigate the biological parents. Surprisingly, all the three children had not 35delG, which is the most common GJB2 mutation.
These results highlight the usefulness of Cx26 mutation screening for genetic counseling and suggest importance of entire sequencing of the gene responsible for DNFB1.

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Interactions between myosin VI and snapin in the sensory hair cell

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Myosin VI, a member of the myosin superfamily with the unique property that it travels along actin filaments toward the pointed end, is responsible for deafness in humans and mice. In mammalian cells, myosin VI is involved in endocytosis, in maintenance of Golgi complex morphology and secretion, and in membrane ruffling. In the inner ear, myosin VI is expressed solely in the sensory hair cells. In the Snell's waltzer (sv) mouse, an intragenic deletion leads to truncation of the myosin VI gene, leading to a null mutation. These mice exhibit circling, head-tossing, hyperactivity and are deaf. In hair cells of normal mice, myosin VI is found in the pericuticular necklace and at the base of the stereocilia. Using the yeast-two hybrid system with a portion of the myosin VI tail as bait, we identified snapin as a putative interacting partner. Snapin has been implicated as a synaptic vesicle membrane protein in neuronal cells and as a soluble factor in non-neuronal cells. The interaction of snapin with myosin VI was verified by a GST pull-down assay, using GST-snapin and incubated with total cochlear extracts, and a co-immunoprecipitation assay in HEK293 cells. Immunoflouroscent staining with specific antibodies against snapin showed co-localization with myosin VI in the cuticular plate of the cochlear sensory hair cells of wild type mice. Our hypothesis is that the myosin VI-snapin complex is involved in endocytosis and/or vesicle trafficking in the hair cells.

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A neural field approach for the study of tinnitus decompensation

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Tinnitus is one of the most common symptoms affecting people all over the world. Unfortunately, till now there is no established cure partly due to a lack of knowledge in regard of its associated comorbidities mechanisms. In this respect, we propose a mean field model of selective attention neural correlates so as to gain deeper insight into the relationship between selective attention and the tinnitus decompensation with respect to large-scale neural correlates. Simulations carried out by using our framework were in close agreement with current tinnitus therapy approaches as well as leading models. It is concluded that our approach represents a step ahead toward the establishment of a reliable simulation framework for clinical settings.

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Biochemical changes found in some inborn errors of metabolism in order to understand visual impairment; homocystinuria-case presentation

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The group of inborn errors of metabolism (IEM) corresponds for a field of pathology that helps in understanding the molecular and biochemical mechanisms in many organ functioning, mainly the brain.
Out of many IEM, homocystinuria and some mitochondrial disorders have the clinical presentation marked by visual impairment (explained today by different mechanisms), beside other specific features. Cases presentations in this field of pathology are very important in order to understand the molecular and biochemical changes that explain these abnormalities in which phenotypes are similar but not identical.
The case presented correspond for a 3 years old child with visual progressive impairment and the clinical and biochemical investigations are suggestive for a genetic disease; the biochemical investigation using the two dimensional thin layer chromatography methodology used in our laboratory provides a peculiar aspect for CBS deficiency (with changes in homocysteine and methionine levels).
Homocystinuria due to Cystathionine β synthase (caused by mutations of the fibrillin-1 gene) is a metabolic disease with a wide spectrum of clinical abnormalities, in which all the patients have eye involvement (dislocation of the ocular lens, retinal degeneration and atrophy, myopia, glaucoma) beside the mental retardation, seizures, thromboembolic complications that are occurring in arteries and veins.
This deficiency could be treated and several countries carry out neonatal screening for CBS deficiency. The treatment includes a low-methionine diet, vitamin B6, folic acid, and betaine (N-trimethylglycine). Some cases are pyridoxine responders and when the low-methionine diet was started in the newborn period, the start and progression of lens dislocation, retinal atrophy were delayed, the mental retardation was prevented and the incidence of seizures decreased.

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Tonotopic' contribution of Ca2+-sensitive potassium channels to cholinergic inhibition of cochlear hair cells

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In the mature cochlea, outer hair cells (OHCs) receive a prominent cholinergic efferent innervation from the superior olivary complex that suppresses cochlear sensitivity. This inhibitory effect is mediated by a tight coupling between a highly calcium permeable alpha9/10 containing nicotinic ACh receptor (nAChR) and the calcium-dependent potassium channel SK2. Recent immunohistochemical data have suggested that, in addition to SK2, OHCs also express the low affinity calcium sensitive BK channel. Interestingly, while they are extrasynaptically localized in IHCs, BK channel expression is restricted to the basal pole of OHCs, suggesting that they may play a role in efferent inhibition. To further investigated the expression and sub cellular localization of BK channels in OHCs and to test whether they are involved in the OHC efferent inhibitory response, we performed a variety of experiments using immunofluorescence and patch-clamp electrophysiology on OHCs in either low frequency (LF) or high frequency (HF) regions of the organ of Corti (19 to 21 days old rat).
Confocal microscopy of organ of Corti immunolabeled with antibodies specific for the BK channel and for markers of either the OHC (prestin and CtBP2) or the efferent neurites (NaK-ATPase, synapsin) verified the localization of BK channels to the basolateral membrane of the OHCs, specifically postsynaptic to the site of efferent contact and, enabled us to further quantify their tonotopic expression. While very few apical OHCs express the BK channel, a high percentage and virtually every OHCs efferent contact are associated with BK immunolabel in the middle and basal turn respectively. Consistent with the BK immunolabeling, specific BK channel blockers applied to apical OHCs had little or no effect on K+ currents activated by either membrane depolarization or local application of ACh. In contrast, both voltage and ACh-gated K currents in basal OHCs were significantly blocked by IBTX and other BK blockers.
This combined approach demonstrates for the first time a correlation between BK channel synaptic localization and its relative contribution to efferent inhibition along the cochlea's tonotopic axis.

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