Lectures

Clinical Trials of CNTF for retinitis pigmentosa

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Retinitis pigmentosa (RP) refers to a set of neurodegenerative diseases that cause progressive loss of photoreceptor cells leading to vision decline and ultimately blindness. RP has a complex genetic etiology, with over 160 causative genes identified over the past two decades. Since the 1990s, neurotrophic factors have shown efficacy in rescuing photoreceptor cell death in many animal models of retinal disease. Recent studies have focused on the potential use of these factors to preserve human vision.

Delivery of large therapeutic proteins to the retina is limited by their inability to penetrate the blood-retina barrier. New cell-based delivery technologies offer promising approaches to this challenge. One example is human RPE cells transfected with the human ciliary neurotrophic factor (*CNTF)* gene and grown on a scaffold of polyethylene fibers within a semi-permeable membrane with sealed ends. CNTF is released from this encapsulated implant into the vitreous through 15 nm pores. The 6 mm by 1 mm devices are surgically implanted into the eye providing a continuous supply of CNTF for many months and possibly for years.

We designed and conducted a Phase I open-label, non-randomized, six month human clinical study to evaluate the safety of CNTF delivered through encapsulated cell implants in 10 subjects with retinal degenerations. Five subjects received lower-dose implants, and five subjects received higher-dose implants. All subjects had visual acuity less than 20/100 in the implanted eye, and visual fields were constricted to less than 40 degrees to the V4e target on the Goldmann perimeter. Three patients had extremely limited baseline acuity, and while those patients reported no diminishment of visual perception, this could not be verified with formal visual acuity measurements. Of the remaining seven patients, three showed improved visual acuity gains of $\geq$10-letter improvement which were evident 6 months after implant removal. Mean acuity of the fellow, control eyes was unchanged during the course of this study. The Phase I CNTF trial demonstrated safety of gene-based intraocular delivery, and provided the basis for Phase II/III human clinical trials. These randomized, double masked, sham-controlled, multi-center trials are testing the effect of CNTF on 170 patients with either early or late stage RP or macular degeneration. In all trials, a change in retinal thickness was observed 12 months after implantation. These results are preliminary, and data collection, analysis and follow-up are ongoing.

Neuroprotective effect of antiapoptotics and antioxidants as a therapeutic option for retinitis pigmentosa

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Hereditary retinal degenerations initiated by gene defects in cones, rods or the retinal pigment epithelium often trigger a neurodegenerative process ending in a loss of the sensory retina and vision. The neural retina responds to this challenge by cellular death and remodeling, first by subtle changes in neuronal structure and later by large-scale neuronal circuitries reorganization. Retinal degenerations in the mammalian retina are accompanied by glial and vascular changes. Part of the ongoing neuronal death could arise from a persistent deficiency in oxygen and nutrient supply. The mechanism of cell death of rods and other retinal cells in retinal degenerations is poorly understood. Oxidative damage, apoptosis and inflammation are the hypothetical pathways involved in degeneration. During the apoptosis process elevated levels of intracellular Ca2+, mitochondrial dysfunction and generation of reactive oxygen species (ROS) have been reported. At present there is no effective treatment for retinitis pigmentosa. However, there are several alternative therapies that are currently being investigated in order to slow or cure this disease. Gene therapy, encapsulated cells and stem cell transplantation are hopeful future strategies towards its treatment. Meanwhile, it would be interesting to study other possible treatments in the short term, at least that could delay retinal degeneration.

Among the strategies used to slow the degeneration of photoreceptors is the use of neurotrophic factors, antiapoptotics and antioxidants that could promote neuron survival and delay retinal remodeling. The purpose of this project is to evaluate the potential of natural compounds as therapeutical agents.

Tauroursodeoxycholic acid (TUDCA) is a compound with antiapoptotic and neuroprotective effects on mouse model of retinitis pigmentosa. On the other hand, it has been reported that prefeeding rats with saffron (an extract from *Crocus sativus*) provides protection against photoreceptors death induced by exposure to continuous bright light. The aim of this study was to evaluate the effect of both substances on photoreceptor degeneration, synaptic connectivity, functional activity and retinal capillary network in the transgenic P23H rat model of retinitis pigmentosa.

Treatment with TUDCA or safranal slows photoreceptor cell degeneration and prevents the loss of retinal function, preserving cone and rod structure and function, together with their contacts with postsynaptic cells. Besides, they prevent the disruption of vascular network and of their associated astrocytes, taking place in P23H rats. TUDCA is also able to avoid the mitochondrial degeneration that occurs in the P23H rat. This study suggests that the neuroprotective effect of TUDCA and safranal could be useful for the future treatment of neurodegenerative diseases.

The use of aminoglycosides and their derivatives as a therapy for Usher syndrome

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Usher syndrome (USH) is a recessively-inherited condition, characterized by the combination of hearing loss and retinitis pigmentosa (RP). RP is a degenerative disease of the retina, characterized by gradual restriction of the visual field, eventually leading to blindness. While the auditory component of USH can be treated by hearing aids and cochlear implants, to date there is no effective treatment for RP. The most severe USH form is USH1, characterized by congenital and profound deafness and prepubertal onset of RP. USH1 can be caused by mutations of at least five genes. While truncating mutations of these genes cause USH1, missense mutations of some of the same genes cause only nonsyndromic deafness. These observations suggest that partial or low level activity of the encoded proteins may be sufficient for normal retinal function, although not for normal hearing. Interventions to enable at least some translation of full-length protein, may delay the onset and/or progression of RP in individuals with USH1 due to nonsense mutations. One such possible therapeutic approach is suppression of nonsense mutations by small molecules such as aminoglycoside antibiotics and their derivatives. This strategy is being tested for several genetic diseases with encouraging results. We are examining this approach as a potential intervention for vision loss in patients with USH1 due to nonsense mutations. Using an *in vitro* transcription/ translation assay of a reporter plasmid we demonstrated efficient suppression of various nonsense mutations of *CDH23, PCDH15,* and *USH1G* (underlying USH1D, USH1F, and USH1G, respectively) with the commercially available aminoglycosides paromomycin and gentamicin. We also obtained *ex vivo* suppression of nonsense mutations with the same aminoglycosides. Nevertheless, a major limitation in the use of commercially available aminoglycosides in humans is their marked toxicity in mammals, which is mainly manifested as nephrotoxicity and ototoxicity. In an effort to address this problem, we have designed and synthesized new aminoglycoside-derivatives, and searched for new compounds with improved stop codon read-through activity and lower toxicity in mammalian cells. Two of these new aminoglycoside derivatives are NB30 and NB54. *In vitro* and *in vivo* assays, together with cellular and acute toxicity experiments, proved both a suppressive activity and a significantly reduced toxicity of these compounds in comparison to commercially available aminoglycosides. The research described here will have important implications for development of targeted interventions that are effective for patients with USH and nonsyndromic RP caused by various nonsense mutations.

Gene therapy for Usher syndrome type 1B

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Usher 1B is caused by mutations in *MYO7A*, which encodes an unconventional myosin consisting of >2000 amino acids. Mutant phenotypes have been identified in the RPE and photoreceptor cells of MYO7A-null mice. They include mislocalization of RPE melanosomes and photoreceptor opsin, the correction of which can be used to assay the efficacy of introducing functional MYO7A into each of the two cell types. The phenotypes of each cell type occur independent of those in the other, indicating the necessity of treating both cell types in Usher 1B gene therapy. Initial studies were carried out on MYO7A-null mice, using HIV-1 derived lentiviral vectors. This treatment proved to be efficacious, although the level of *MYO7A* expression varied from cell to cell, most likely because of different integration sites. We now report effective treatment with a non-integrating HIV-1 lentivirus: subretinal injection results in *MYO7A* expression and correction of mutant phenotypes in the photoreceptor and RPE cells. The advantage of the non-integrating lentivirus is that expression is comparable from cell to cell, and the risk of insertional mutagenesis is avoided. In addition, we have found that AAV2 vectors can deliver functional MYO7A to photoreceptor and RPE cells, despite the carrying capacity of these vectors being limited to ~5.4 kb. AAV2 has now been used successfully in gene therapy clinical trials for treating loss of function of RPE65, however, due to the large size of the *MYO7A*, the AAV vectors must be "overstuffed" in order to deliver this gene. Our results indicate that, for a given titer, serotypes 2 or 5 were equally efficacious in terms of MYO7A levels and mutant phenotype correction. Use of non-integrating lentiviral-*MYO7A or* AAV2-*MYO7A* therefore appears to be a viable approach for treating the retinal degeneration of Usher 1B.

USH1C therapy strategies in the retina

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The Usher syndrome (USH) is an autosomal recessive disorder. Usher syndrome type I (USH1) is the most severe subtype with congenital severe to profound hearing loss and pre-pubertal onset of *retinitis pigmentosa*. The *USH1C* gene encodes the PDZ containing scaffold protein harmonin which is expressed in form of numerous alternative spliced variants. Hamonin is one of the key organizers in the USH protein network directly binding to all USH1 und USH2 proteins.

Currently only the amelioration of the hearing deficiency symptoms by cochlear implants is implemented, but no treatment of the senso-neuronal degeneration in the eye exists so far. The postnatal onset of retinal degeneration and good accessibility of the eye makes the retina an approachable therapeutic target.

We currently assess different gene-based therapy options: The straight forward strategy of gene addition using non-viral or viral vectors. Recent success in the reversion of blindness by recombinant adeno-associated virus (rAAV) mediated gene transfer in humans affected by *Leber congenital amaurosis* increased hope for rAAVs as a treatment strategy for USH in the eye. Preliminary data indicate that rAAVs are also suitable for the transfer of harmonin to photoreceptor cells. Nevertheless, various splice variants of the *USH1C* gene with yet undefined expression profiles and functions make gene addition difficult for USH1C. The increasing knowledge on specific mutations causing the USH phenotype enables the development of mutation-specific therapy strategies, namely gene-repair by homologous recombination mediated by zinc-finger nucleases and treatments with translational read-through drugs, e.g. modified aminoglycosides or PTC124. Latter compounds target in-frame nonsense mutations which account for ~20% of all USH cases. In particular, PTC124 yielded promising results in phase II clinical trials for the treatment of various non-ocular diseases caused by in-frame nonsense mutations.

All discussed gene-based therapy strategies should lead to the restoration of the expression of the functional USH1C protein harmonin. These adjustments may be sufficient to cure or at least slow down the progression of retinal degeneration which would greatly improve the life quality of USH1 patients.

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Stem cell therapy for Usher 2a

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Background: We are focused on using cell-based therapy to treat the vision loss associated with Usher's syndrome, the most common cause of deafness and blindness. There is no treatment for the vision loss, which occurs as a result of progressive degeneration of photoreceptors, which reside in the outer retina, adjacent to the retinal pigment epithelium (RPE). The consequence is progressive loss of the field of vision followed by acuity. In humans, mutations in the *Usherin* gene account for approximately 70% of type 2 Usher syndrome and additionally are a frequent cause of non-syndromic retinitis pigmentosa. It is estimated that 10,000-20,000 individuals are affected in the US alone.
Over the past 12 years, we have explored the value of a cell-based therapy approach to slow or halt the progress of photoreceptor degeneration and associated vision loss by introducing cells into the space between the photoreceptors and lining RPE. We have identified a human forebrain-derived cell line (hNPC) that is both safe and maximally effective in sustaining vision and retinal structure.

Purpose: To study the morphological and functional changes in an animal model of human Usher syndrome 2A, the *Ush2a* mouse, and explore the efficacy of transplantation of hNPC in preventing of visual deterioration.

Methods: Ush2a mice were tested from postnatal days (P) 70 to more than P700, using the optomotor test to evaluate rodent visual acuity and sensitivity to changes in contrast. Mice received subretinal grafts of hNPC cells at P80 and were tested for up to 10 weeks post-grafting. At the end of testing, animals were sacrificed and eyes processed for histology.

Results: The optomotor test showed that both acuity and contrast sensitivity deteriorated over time. Photoreceptor loss was only evident later than 1 year of age, although changes in the intracellular distribution of red/green cone opsin were observed as early as P80. Mice who received cell transplants performed significantly better than control mice and did not demonstrate abnormal distribution of cone pigment.

Conclusions: This study showed that vision impairment was detected well before significant photoreceptor loss, and was correlated with abnormal distribution of a cone pigment. Cell transplantation prevented functional deterioration for at least 10 weeks and reversed the mislocalization of cone pigment.

Gene therapy for Usher syndrome type 3

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Usher syndrome type 3 (USH3) is characterized by progressive bilateral sensorineural deafness and retinitis pigmentosa. USH3 patients benefit from hearing aids and/or cochlear implants, while there is no cure for the retinal degeneration.
The causative gene for the USH3 phenotype is *clarin 1* (*CLRN1*), encoding for a 232 amino acid four transmembrane domain protein clarin 1 (CLRN1) with unknown function. The *CLRN1* gene is expressed in several tissues, including inner ear and retina. Careful analysis of the recently generated *Clrn1*$^{-/-}$ mouse has revealed progressive hearing loss and hair cell degeneration, but no histological or functional retinal abnormalities.
Adeno associated viral (AAV)-based gene delivery in several animal models of retinal dystrophies has been shown to be safe and therapeutic. Recent findings have lead to successful human clinical trials in a rare form of childhood retinitis pigmentosa.

Purpose: To evaluate the potential benefits of AAV-based *clrn1* gene delivery in the diseased retina, we designed a study to test AAV-*clrn1* delivery into the *Clrn1*$^{-/-}$ mouse cochlea for possible therapeutic effect.
To search for a suitable AAV gene therapy vector, we studied transduction efficiencies of recombinant AAV2/1, 2/2, 2/5 and 2/8 vectors encoding GFP marker or HA-tagged *CLRN1* in HEK-293 cells and mouse cochlear tissue cultures. AAV2/1 and 2/2 encoding GFP were also studied in adult wt C57B mice. Therapeutic effects of AAV2/2 and 2/8 encoding HA-tagged *CLRN1* were studied in P13-14 *Clrn1*$^{-/-}$ mice by auditory brain stem responses and immunohistochemistry. All vectors were delivered to cochlea by microinjection.

Results: AAV2/1 and 2/2 infected HEK-293 cells more efficiently than AAV2/5 and 2/8. Transduction efficiency of self-complementary AAV2/2 was significantly higher than that of conventional AAV2/2. AAV2/1 and 2/2-mediated GFP expression was present in the organ of Corti of wt mouse 6 days after cochlear injection. AAV2/2-*clrn1*-HA and AAV2/8-*clrn1*-HA infect the cochlear hair cells and the cells express HA-tagged clarin1. About 1/4th of the inner hair cells were infected with the current protocol. One *Clrn1*$^{-/-}$ mouse showed elevated auditory brainstem responses after AAV-*clrn1*-HA injection but did not show substantial hair cell survival. We also discovered that in one animal the infected side showed more hair cells than the un-injected side. This animal was the least mature at the time of injection (P13). However, there is a range in the rate of hair cell loss among these animals.

Conclusions: AAV2/2 and AAV2/8 are suitable viral vectors for future studies with the *Clrn1* knockout mouse. Studies continue with injections of potentially therapeutic AAV-*clrn1*-HA constructs into the inner ears of younger *Clrn1*$^{-/-}$ mice.

State of the art clinical and genetic diagnosis and early intervention in Usher syndrome

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Profound congenital deafness is one of the most common severe disabilities (1-2/1000 newborns). Approximately 30% of these children have syndromal deafness. The most common causes of syndromal deafness are genetic (80-90%) and the most common combination of organ dysfunctions are ear and eye- deafblindness. Among these Usher syndrome accounts for 50%. The possibilities of early diagnosis have today been possible through state of the art clinical and genetic testing. One crucial test is neonatal hearing screening. When a child with profound deafness is diagnosed, a vestibular test is performed (2-3 months). If pathological one very likely diagnose is US 1 and an ERG and genetic testing have to be performed. If US 1 is confirmed, bilateral CI should be offered. The introduction of CI have totally changed habilitation and dramatically increased and diversified activities and participation. In children with moderate to severe hearing loss you have to use signs for early visual deficiencies and genetic screening for US in order to diagnose children early. Universal screening children with different visual tests including ERG is not at this stage recommended. Better knowledge concerning cognitive functions is also at need. Hopefully the “hit rate” with better genetic screening tools will make it possible of earlier and more valid diagnosis. Early diagnosis will even more in the future be essential since it is likely that treatment will be gene specific. The importance of early and correct diagnoses for early intervention will be discussed.

Psychosocial impact of Usher syndrome: Adults and the family

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Usher Syndrome has a significant psychosocial impact throughout life. The signature issues for deafblind people are access to the world and isolation. The diagnosis can be devastating as seen by reports of depression, grief, alcoholic binges. The consequences of visual deterioration are felt in all spheres of life: education, mobility, work, ability to earn, communication, relationships with family, partners, children, and friends, roles in the community, and involvement in community activities.

The concrete losses of work, ability to communicate and travel, troubled relationships combined with the loss of sense of competence, security, and self- worth lead to a feeling that the basic self has been lost.

There are significant feelings of threats to one's identity, sense of self, competence and worth. People with Usher Syndrome report being marginalized in the community of their primary identity, the Deaf community or the hearing world and report feeling they are no longer who they were.

After interviewing more than 100 people with Usher Syndrome over many years, in 2007, the Information Center for Acquired Deafblindness in Denmark and this writer interviewed 10 people with Usher in Denmark and 12 people with Usher in the United States about their lives, their experiences, and their sense of identity. They were asked how they identify themselves, as deaf or hard of hearing with vision problems, or as deafblind, among other choices.

The life experiences and feelings of the two groups, the Danes and the Americans, facing the consequences of Retinitis Pigmentosa, were very similar, in terms of job loss, limitations imposed by Usher, depression, and family relationship upset.

There are significant differences in services available to deafblind people in Denmark and the US; some services available by law in Denmark have no US equivalent, except in largely volunteer form. These services available to Danes who are deafblind provide opportunity for more contact with each other in a variety of community and organized activities and formats and access to the outside world.

Meeting with others with Usher and who are deafblind provides support, leaving a lasting impression and a sense that life goes on, although differently. This seems to have enhanced a positive sense of identity as Deafblind. They allow deafblind people to remain active in the world with family, friends, and activities and to be less dependent on others, all of which improve quality of life.

Usher syndrome and psychosocial health

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This presentation is part of a larger study on Usher syndrome and psychosocial factors that promote health.

The aim is to study psychosocial factors that promote health among persons with Usher syndrome type II and III.

Usher syndrome is an inherited condition that impacts both hearing and vision, it can be separated into three different clinical groups that are named I, II and III. To have Usher type II means that the individual have a congenital mild to moderate hearing loss that is quite stable and a normal balance, the vision problems are constituted by Retinitis Pigmentosa (RP) with an onset in the teens and a progressing course during life. Usher type III means that the person have an acquired profound hearing loss with an early onset and RP as in Usher type II, and progressing balance problems.

To have a diagnose that has a progressive course from the vision involves great consequences on communication and daily life; this can effect the psychosocial wellbeing in a negative way.
Another aspect is the relationship between geno and phenotype, which is the effect of specific mutations on vision, hearing, balance and possibly other organs, not yet known.

Two different questioners have been used, the Hospital Anxiety and Depression Scale (HADS) and the National public health survey that cover physical and mental health among people in Sweden. The HADS is a self –assessment scale that is validated and used for detecting depression and anxiety. The national public health survey contains of approximately 75 questions concerning health, illness and wellbeing, eating habits, sleep, social relations, stress, suicide thoughts/ attempts and background questions. These have been answered by 96 persons (45 men and 51 women) with Usher type II and 16 persons (5 men and 11 women) with Usher type III. The results for the Usher population are compared to a normal Swedish reference population.

The results and consequences of psychosocial factors such as depression, anxiety, sleeping disorders, self esteem, thoughts of suicide, within Usher type II and III will be closely discussed at the work shop. All results are preliminary at this stage, but as far as we have analyzed now there are differences when it comes to depression, anxiety and thought of suicide and suicide attempts between persons diagnosed with Usher syndrome type II and III and normal Swedish population.

This research work contains new research that has not been presented earlier.

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 7.3 Mb large interval.

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Usher scaffold proteins provide complementary functions in retina and inner ear

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The Usher proteins, harmonin (USH1C) and whirlin (CIP98), contain PDZ protein interaction domains and are thought to act as scaffolds that bind other Usher proteins into a macromolecular complex. Although much is known about the physical interactions *in vitro* of the harmonin and whirlin PDZ domains with the other Usher proteins, it is unknown whether these proteins function as scaffolds *in vivo* to organize the Usher proteins. It is also unclear whether the two proteins organize different combinations of Usher proteins into complexes, or whether they carry out redundant or different functions.

We have studied these questions by knocking down the functions of one or more scaffold proteins and analyzing vision, hearing, balance, and localization of other Usher proteins *in vivo*. We use zebrafish for these studies because it is quick and easy to manipulate gene expression and because most available mouse mutants do not show eye defects.

We discovered that the *cip98* gene is duplicated in the zebrafish genome. Both genes encode full-length Cip98 proteins with three PDZ domains. We generated antibodies that specifically distinguish the Cip98 and Ush1c proteins and found that the proteins have distinct and highly dynamic localization patterns in photoreceptors and Müller glia of the retina and in sensory hair cells and neurons of the inner ear.
Mutation of *ush1c* or knockdown by morpholino antisense oligonucleotides produces hearing, balance, and vision defects. In the retina, ribbon synapses of the photoreceptors are disorganized and synaptic transmission is disrupted. In the ear, fewer stereocilia form and the ones that do are bent and splayed. Dye uptake assays indicate that mechanotransduction is defective. We also see mislocalization of other Usher proteins.

Similar to *ush1c*, we find that knockdown of either c*ip98* gene results in hearing, balance, and vision defects. Thus, these duplicates are not functionally redundant. Knockdown of *cip98a* blocks light dependent ciliary transport of opsin in photoreceptors.

These results suggest that harmonin and whirlin act as scaffolds to organize Usher proteins into complexes in vivo, and that the complexes have complementary functions in different cellular and subcellular regions of the retina and inner ear.

The USH2A Interaction Partner NINL^{isoB} associates with BBS6, plays a role in establishing planar cell polarity and functions in cilia assembly

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Usher syndrome (USH) is the most common cause for hereditary deaf-blindness in man. USH is a heterogeneous disorder for which nine causative genes have been identified so far. We have shown that the corresponding USH1 and USH2 proteins interact and that they function in a (dynamic) protein complex at different subcellular sites in the inner ear and retina. Defects of members of the USH protein network can lead to malfunction and degeneration of both hair cells and photoreceptor cells by mechanisms that are not yet fully understood. To elucidate the pathogenic mechanism underlying Usher syndrome, we searched for novel interacting partners for the intracellular region of USH2A isoform B.

Protein-protein interaction assays identified a novel isoform of the centrosomal ninein-like protein (NINL^{isoB}) as a member of the USH protein complex. In order to decipher the role of this protein *in vivo*, knockdown studies in zebrafish were performed. These gave rise to a classical planar cell polarity (PCP) phenotype, similar to the defects observed after knockdown of genes involved in Bardet-Biedl syndrome (BBS). Furthermore, strong genetic interactions were found between *Ninl* and each of the *Bbs* genes, indicating that Ninl and the Bbs proteins indeed function together in the PCP pathway. A physical interaction could only be demonstrated between NINL^{isoB} and BBS6.

The non-canonical (PCP) and canonical Wnt signaling pathways are closely connected and co-regulated. TOPflash reporter assays showed that downregulation of *NINL* also interferes with the canonical Wnt signaling pathway. Extracellular Wnt signals are captured by cilia and transferred to the intracellular environment of the cell. Interestingly, we identified that reduced *NINL* expression in ciliated hTERT-RPE1 cells lead to defects in cilia assembly, which might be the primary cause of the defects observed in zebrafish.

Our data indicate that the same centrosomal protein interacts physically with USH2A and BBS6, thereby linking the ciliopathies Usher syndrome and Bardet-Biedl syndrome at the molecular level, which is indicative for an overlap in pathogenic mechanisms underlying these disorders. Our data support previous indications for a putative role of the Usher interactome in the establishment of planar cell polarity and provide indications for the underlying molecular mechanisms with a central role of cilia.

How do people with Usher syndrome live their lives?

A five-year Nordic research project gathering experiences from people with Usher syndrome

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”The Nordic Project” is a scientifically based research project of the personal experiences of people with Usher syndrome and how they handle the many challenges they are facing. Over a five-year period 20 people with Usher syndrome (type 1 and type 2) in Denmark, Norway, Sweden and Iceland were interviewed once a year by a deafblind consultant.

The project was completed by the publishing of six booklets – each focusing on an individual topic – with the following titles:

- Theory and methods
- Receiving a diagnosis
- Getting support
- Being active
- Getting an education and work
- Narratives of everyday life

Much too often, significant research results end up in a report on a shelf. There the report sits comfortably, but without having any real impact for the people which the research is about. This project was meant to have a practical impact on actions and decisions in the field – not because it holds any specific instructions on what could or should be done, but because it lends a voice to a multitude of statements about what it is like to live with a progressive vision and hearing impairment. One of the deafblind participants said regarding his involvement in the project:

"A social counsellor only works with problems, for example transport. We never talk about my everyday life, my routines and my network. Before this project, I have never spoken about those problems either to a psychologist or to a social counsellor. They just look for a problem and then talk about it over and over again."

In this workshop we will introduce the project and its outcome. What does it tell us about the lives of people with Usher syndrome, and what can this knowledge be used for – also in countries outside Scandinavia?

The project was initiated and managed by the Information Center for Acquired Deafblindness.

The booklets (in English) will be handed out during the presentation.

Deafblindness and the notion of trust, ontological security, social recognition and self-identity

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In the presentation a psychological dimension (trust and ontological security) and a psychosocial dimension (social recognition) are discussed in relation to self-identity among people with acquired deafblindness. Trust and ontological security are closely related and they have been discussed in relation to e.g. schizophrenics. However the phenomenon has not been elaborated in the context of deafblindness. In interaction with the social and material environment reliability, constancy and predictability are crucial. When these relations fundamentally change the impact on the ontological security will be very negative. Furthermore, among the basic human needs are to be recognised as a human being. The construct of recognition is embodied across three dimensions: individual (self-confidence), legal (self-respect) and value (self-esteem). The interaction between trust and ontological security on the one hand and social recognition on the other hand is discussed and illustration from an ongoing study of persons with Usher type II are presented. In the final part of the presentation it is argued that these basic processes of personality development has to be paid attention to and acknowledged in interaction with people with deafblindness. Some implications for rehabilitation of people with acquired deafblindness are suggested.

Berth Danermark is professor in Sociology at the Swedish Institute for Disability Research, Örebro University and Linköping University, Sweden. In his research he focuses psychosocial aspects of disability, e.g. deafblindness. With colleagues at the Institute he has developed a metatheoretical approach to disability based on the notion of bio-psycho-social approach to disability advocated among others by WHO. He has also conducted research focusing inter-professional cooperation in relation to people with disabilities. Berth.Danermark@oru.se

Lessons from the UK National Collaborative Usher study

Maria Bitner-Glindzicz[1], on behalf of the NCUS*
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The UK NCUS is a genetic and phenotypic study of 190 families with Usher syndrome in the UK. All known Usher genes were sequenced in all patients and all were offered full audiovestibular and ophthalmic phenotyping regardless of their predicted clinical subtype.

Of the 45 patients who had an initial clinical subtype of Type 1 Usher syndrome, the molecular basis was determined in almost 80% by sequencing the known genes *USH1B/MYO7A, USH1C/USH1C, USH1D/CDH23, USH1F/PCDH15* and *USH1G/SANS* and confirmed by Sequenom MassARRAY in probands, families and controls.

Pathogenicity was ascribed to nonsense mutations, insertions/deletions leading to frame shifts, splicing mutations and large deletions and to previously published missense mutations that were not present in 870 control chromosomes, are pathogenic according to *in silico* studies and segregate with the disease phenotype. "Likely pathogenicity" was ascribed to novel missense mutations which segregate with the disease which are not present in 870 control chromosomes and were detected *in trans* with a certainly pathogenic mutation.

We identified a number of sequence artefacts which have still to be resolved, and some 'atypical' cases including siblings with a 'type 3-like' audiovestibular phenotype and an unusual presentation of RP, but who had mutations in *USH1C*, and a case in which a child appears to have a de novo mutation in *MYO7A*.

Sequence analysis of 'non-Type 1' patients for *USH2A*, *VLGR1*, and USH3A genes is nearly complete. No convincing examples of digenic inheritance have been found to date (ie. two definitely pathogenic mutations in different genes), although a number of missense variants are present in different genes, particularly in patients from minority ethnic groups.

Development of the ERG in the first five years of life

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14 children (range 2 – 10 years of age) with different genetic types of Usher syndrome were examined with full-field electroretinography (ERG) during general anaesthesia for evaluation of the retinal dysfunction during early childhood. Five of these children were repeatedly examined over a period of 5 to 10 years with full-field ERG and these results were compared to full-field ERG data from 58 children without retinal eye disorder, examined under the same conditions.

In addition retinal function in three families with affected siblings with different type of Ushers were further analyzed regarding intra familiar variation of retinal dysfunction.

Full-field ERG demonstrated early alterations in both cone and rod function corresponding to a rod – cone dystrophy in all children. A remaining rod function could be verified in the majority of the children up to 4 years of age. After 4 years of age there was a further deterioration of the rod function, and the progress was severe in Usher type 1 and 2 and moderate in Usher type 3. Three siblings shared the same mutation for Usher 2C demonstrate in contrast to previous reports diverged phenotype and in one of these siblings only moderately reduced rod and cone function. Evaluation of retinal degeneration according to genotype and phenotype in the aspect of total retinal function, enhance our possibilities to characterize the clinical phenotype and to evaluate the pathogenesis in patients with different types of Usher syndrome.The ERG results from the 58 children without retinal disorder fortify that full field ERG during general an aesthesia is a reliable method.

Retinal disease expression in Usher syndrome

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Usher syndrome (USH), a debilitating group of dual-sensory diseases, has entered a list of autosomal recessive disorders that may be amenable to retinal gene therapy. USH1B due to *MYO7A* mutations, one of the subtypes targeted for treatment, has no animal model that manifests retinal degeneration. The onus is thus upon human studies of molecularly-identified USH1B patients to define the retinal disease expression and determine how to approach this entity for treatment.

We use *in vivo* retinal imaging by optical coherence tomography and photoreceptor-based psychophysics to define the microanatomy and visual function in USH1B. Conclusions are being drawn from the results about what specific retinal regions are worth targeting for treatment and for monitoring in early phase trials.

Vestibular function in children with Usher syndrome: What do we know and how should we study it?

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Usher syndrome (USH) is a heterogeneous recessive genetic syndrome resulting in sensorineural hearing loss (SNHL), retinal degeneration leading to blindness (RP, retinitis pigmentosa), and vestibular dysfunction. It affects up to 20% of infants with congenital SNHL, 15-30% of patients with RP, and 50% of deaf-blind children. Vestibular dysfunction in USH was originally thought to be central in origin, and abnormalities of the cerebellum have been reported in the past. More recent studies in both humans and animal models with USH support peripheral vestibular abnormalities, with the vestibular hair cells, utricle and saccule, being affected. Although the presence of vestibular dysfunction in a child with SNHL could raise the suspicion of USH, balance abnormalities are often not appreciated by either parents or clinicians. This is compounded by the fact that vestibular testing in infants and young children is not standardized, not readily available, and not easy to perform. The few clinical studies have concentrated on patients with USH1, who are late walkers and therefore the most obviously affected. However, marked genetic heterogeneity and the recognition that the USH proteins often function in an "interactome" suggests that patients with all three forms of USH could have vestibular abnormalities. Finally, genetic testing for USH is not always available, often not covered by insurance, and results may be difficult to interpret. Classification of the vestibular abnormalities of USH, taken together with the SNHL and any eye findings, would strengthen a possible diagnosis of USH, support genetic testing, and enable the patients and families to plan a comprehensive rehabilitation plan.

We hypothesize that vestibular dysfunction can be detected in even very young children with USH. Our aim is to identify assessments of vestibular function that can be performed in an outpatient setting, and that will: 1) support a clinical diagnosis of USH; 2) support pursuing genetic testing; 3) guide clinical management. Children with bilateral SNHL and biallelic pathogenic mutations in any of the USH genes will undergo vestibular testing and assessment of gross motor development. Test methods include: 1) standard neurologic physical examination (finger to nose, Romberg, past pointing) for patients >42 months; 2) assessment of gross motor function using the Bayley Scales of Infant and Toddler Development, 3rd Edition (Bayley, 2006) for patients < 42 months; 3) Vestibular evoked myogenic potentials (VEMPS); 4) Subjective visual vertical (SVV); 5) Review of brain and temporal bone MRI and CT, when available, to assess for brainstem abnormalities. Analysis of results will include genotype-phenotype correlation, including hearing loss and ophthalmological finding.

To date, sixteen patients with biallelic USH mutations have been identified for study: 9 with USH1 ([8]MYO7A, [1]CDH23), 5 with USH2A and one with USH3. Of the 5 with MRI's, 2 have abnormalities: one has a Chiari 1 malformation and another has a poorly characterized cerebellar lesion. The 9 USH1 patients walked between 17 and 31 months. Results for VEMPS, SVV and Bayley scales will be presented.

A DNA based screening test for Usher syndrome

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Usher syndrome is the primary cause of combined deaf/blindness in the United States and Europe. It is a recessively inherited disorder due to mutations in at least 11 genes, of which nine have been identified at the DNA level. The hearing loss is most often congenital and can be either profound (deaf) or moderate (hard of hearing) while the retinitis pigmentosa is a progressive loss of rod function manifested first by nightblindness and loss of peripheral vision, and ultimately, in many cases, blindness as an adult. The early diagnosis of Usher syndrome promises many benefits for early rehabilitation and education and would provide subjects for natural history studies in children. However, traditional diagnostic tests like the electroretinogram (ERG) are difficult and expensive to perform on young children. We have developed an inexpensive Usher screening test. The test is directed against recurring mutations observed in all known Usher genes. A positive result requires follow up to confirm the mutation by conventional DNA sequencing and clinical testing of retinal function is also required to confirm the diagnosis at the phenotypic level. In most cases, a search for a second mutation will be required. This tiered approach to Usher screening is an inexpensive and feasible mechanism for screening large numbers of children at a reasonable cost. Children with a hearing loss are a high risk group for Usher syndrome. Our studies indicate that more than 10% of children with a hearing loss may have Usher syndrome and develop serious vision problems due to retinitis pigmentosa at a later age. We are now developing programs to integrate Usher testing with newborn hearing screening programs in four states in the Midwest USA and plan to test older children in State supported special education programs for the deaf and hard of hearing. Early Usher syndrome diagnosis has a great potential for benefit to families and children involved. An Usher screening program will allow for natural history studies to identify differences in the development of RP on a genotype specific basis and would lay the foundation for future clinical trials by providing much needed information on appropriate endpoints as well as potential subjects. Several ideas about treatment to retard the development of the RP have been proposed; it is sensible to believe that early intervention would result in the greatest therapeutic benefit to the Usher patient.

The utility of databases in diagnosis

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With the increasing number of different variants identified in each gene, the creation of centralized and regularly updated databases has become crucial. Locus Specific mutation DataBases (LSDBs) are related to genes and their variations and are now recognized as the best mode of collecting and curating lists of mutations related to human genetic diseases. Complete and accurate data on variants and their effects on the phenotype is essential for proper genetic counselling, and the usefulness of LSDBs is now well established for both clinicians and researchers. Curation of nucleotide variations in individual genes by experts can provide accurate information to the user, both on published and unpublished mutations and polymorphisms. Indeed, while in central core databases (NCBI, Ensembl…) variations are often added via automated processes (scanning of dbSNP…), in LSDBs each variation is added and/or validated by an expert. Therefore the quality of the data, such as nomenclature, effect, or comments, is naturally enhanced.

LSDBs dedicated to the 9 Usher genes have been developed. These LSDSs record so far any published alteration which represents more than 4500 entries corresponding to > 900 unique variants described in more than 1500 patients.

Each variant is recorded following HGVS recommendations, at the nucleotide and protein level, and the last RefSeq entries (for which the NCBI solicited database curators) were used to define the nomenclature.

To date, more than 270 sequence variants of unknown pathogenic effect, so called Unclassified Variants or UVs have been identified among the Usher genes. Assessing their clinical significance represents a challenging task. In addition, because large-scale sequencing is susceptible to represent the future for molecular diagnosis of Usher syndrome, it will generate an increasing number of new UVs. We are currently concentrating our efforts to provide an updated tool dedicated to this aspect that will be accessible through USHbases.

The substantial amount of data already recorded in these databases, together with future developments, make USHbases a unique resource that will continue to assist diagnostics, clinical and molecular research in the field of Usher syndrome.

Epidemiology of Usher syndrome

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The EsRetNet Retinal Network was set up by 6 teams, since 1991: two from Barcelona, one from Seville, one from Valencia, one from Vigo and one from Madrid, working in a coordinated multicentric and multidisciplinary way focused on clinical, epidemiological and genetic research on Retinal Dystrophies (RD).

Up to now, we have registered about 3,000 Spanish RD families and more than 4,500 Spanish affected cases. Among them, we have observed 25 % of Syndromic RP families (N= 601) and more than 400 Spanish Usher families. They represent around 21% of our Spanish RP studied families. 33% of families showed Usher type 1, 62% type II and 4.4% were unclassified Usher families.

The estimated prevalence of Usher syndrome in Spanish population was 4.2/100,000 (ranging from 1.8 to 6.2/100,000) for the region of Valencia (Espinós et al, 1998).
In our population like in others, the most commonly mutated gene in USH1 cases was MYO7A (USH1B), which represents around 45% of the USH1 patients, followed by CDH23 (USH1D), (20-25%) CDH15 (USH1F)(15-20%) being very less frequently mutated harmonin (USH1C), and SANS (USH1G). Among the three genes implicated in USH2, mutations in the Usherin gene (USH2A) account for 70–80% of the USH2 Spanish cases.

Recently, we validated the genotyping microarray Asperbio for Usher in our population, prior to its implementation for genetic diagnosis (Jaijo et al; 2010). We found 33.9% of mutated families (62/183), 31.4% of USH1 patients, 39.4% of USH2 patients, 22.2% of patients with USH3 and 15.8% of patients with unclassified Usher syndrome with a total rate of 26.5% (96/366) pathologic alleles detection The USH2A mutations p.C3267R and p.T3571M were revealed as common in the Spanish population.

Regarding clinical aspects Usher I and II Spanish patients were ascertained for these studies at mean ages of 30.5 ± 13.6 and 35-7 ± 11.5 years respectively. The onset of night blindness and visual field constriction symptoms were at the end of the first decade of life for Usher I and at the end of the 2nd decade for Usher II. These differences were statistically significant. As reported in other series, evolution was also more severe in Usher I than in Usher II, since decreased visual acuity (below 1 in 10) appeared earlier in type I , but these differences were not significant.

We will present these molecular, clinical and epidemiological results in Usher Syndrome Spanish population as well as in other RD related conditions.

Clinical and molecular results allowed us to improve both genetic counseling and clinical management of our patients, as well as to design better health care programs for prevention of these cases. In addition, future gene therapy will be possible in those well genetically characterized cases.

Developing more comprehensive genetic screening strategies for congenital sensorineural hearing loss

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Sensorineural hearing loss (SNHL) is the most common sensory deficit in developed countries. In the United States, for example, about 4,000 infants are born each year with severe-to-profound bilateral SNHL and another 8,000 infants are born with unilateral or mild-to-moderate bilateral SNHL. These figures mean that SNHL is diagnosed three times as frequently as Down Syndrome, six times as frequently as spina bifida and more than 50 times as frequently as phenylketonuria. By etiology, more than half of neonates with SNHL have inherited hearing loss. Most frequently, the inheritance pattern is Mendelian recessive (75-80% of cases), although autosomal dominant congenital SNHL also occurs (~20%). Fractional contributions are made by X-linked (2-5%) and mitochondrial (~1%) inheritance.

Based on the premise that early identification improves speech and language and decreases the expected lifetime cost of SNHL, Universal Newborn Hearing Screening (UNHS) programs have been established in many countries with screening incorporated into early hearing, detection and intervention (EDHI) programs. As a result of these programs, the average age of detection of children with SNHL has dropped from 12-18 months to 6 months or less. Results of these programs suggest that they facilitate normal language achievement for more children with SNHL and therefore offer a cost savings in the long-term compared with both no screening and selective screening.

Screening programs for congenital SNHL have focused on physiologic tests to determine the functional status of the auditory system. These tests include auditory brainstem response testing (ABR, also known as BAER, BSER) and otoacoustic emission (OAEs) testing. Genetic testing has generally played a secondary confirmatory role, with mutation screening offered for a number of different genes. This approach has been very successful, but as habilitation options for hearing loss develop, more comprehensive genetic testing for SNHL will be required. In this talk, we explore developing strategies that permit comprehensive screening for all types of SNHL.

Modifiers of ciliary disease

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Defects of the primary cilium and its anchoring structure, the basal body, cause a number of human genetic disorders, collectively termed ciliopathies, since they are characterized by an overlapping range of phenotypes that include retinal degeneration, polydactyly, renal and hepatic fibrosis, obesity and a complex range of cognitive and neurodevelopmental defects. Recent data have also shown that some ciliopathies overlap not only phenotypically, but also genetically by contributing epistatic alleles that can modulate the phenotypic expressivity and penetrance. As such, the primary cilium and its associated signaling represents a useful model to understand the mechanism of total mutational load in a biological system. Towards that end, we have initiated systematic sequencing and functional evaluation of mutations of ciliary genes in a range of ciliopathy phenotypes and, using a large allelic series, have constructed models of epistasis in oligogenic disorders that suggest an intricate interaction between rare and common alleles to modulate penetrance and expressivity. Such studies will ultimately empower the predictive nature of the genotype and inform clinical management and treatment.

Raymond Iezzi

Mouse models of retinal degenerations: therapeutic interventions and clinical correlations

Drack A, Mullins R, Thompson S, Kinnick T, Bugge K, Nishimura D, Stone E, Sheffield V

Purpose: To characterize the ocular phenotype in mice models of retinal degeneration and to attempt therapeutic interventions.

Methods: M390R *Bbs1* knock-in mice and *Bbs3* knock-out mice were generated as described previously. Pupillometry, electroretinography, fundus examination and photography, and retinal histology including retinal antibody staining were performed in young and old mice. A gene replacement therapy protocol using subretinal injection of AAV viral vector with wild type *Bbs1* was developed. A protocol was developed in which *Bbs* mice, as well as other mouse models of retinal degeneration, are raised in low vs. ambient light, treated with subconjunctival or intravitreal steroid, and treated with systemic TUDCA to determine which interventions modify the retinal degeneration. Clinical correlations to human disease are explored.

Results: M390R *Bbs1* knock-in mice demonstrated abnormal pupillary responses from an early age. Electroretinogram amplitudes were diminished starting at age 3-4 months and progressing to almost non-recordable at 6-9 months. *Bbs3* knock-out mice demonstrated almost non-recordable electroretinograms at 7 months of age and older. A distinct lobular pigmentary pattern was present. Optic atrophy developed, likely due to hydrocephalus rather than the retinal degeneration. Subretinal injection of reporter genes and AAV-WT gene constructs was performed in *Bbs1* mice. Short term electrophysiology and fundus examination demonstrated no adverse effects to the retina compared to the uninjected eye. An injected eye demonstrated rod staining with BBS specific antibody.

Conclusions: Knock-in mice with the most common genetic subtype of Bardt Biedl Syndrome 1 (*BBS1*) in humans, M390R, and a knock-out of *Bbs3* recapitulate the retinal degeneration seen in humans. Several treatment protocols are being tested. Knock-out mice with *Bbs3* develop a marked pigmentary retinopathy as well as hydrocephalus and optic atrophy. Several mice models of retinal degeneration exist which are amenable to testing therapeutic interventions which may provide insight into human retinal degenerations.

Non-invasive evaluation of hearing in mouse models of deafness: a focus on IGF-I deficiency

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Insulin-like growth factor I (IGF-I) is fundamental for the regulation of cochlear growth, differentiation and metabolism, and its mutations are associated with hearing loss in mice and men. IGF-I and its high affinity receptor IGFR1 are expressed in specific spatiotemporal patterns in the cochlea during development. Peak expression of IGF-I occurs during the late embryonic and neonatal periods, being reduced in the adult, a trait that associates with age-related hearing loss.

IGF-I/IGFR1 actions are mediated by intracellular signaling networks primarily activated by the phosphorylation of insulin receptor substrates (IRS2) and down-regulated by the tyrosine phosphatase PTP1B, IRS2 activation leads to the sequential phosphorylation of lipid and protein kinases (c-RAF). IGF-I/IGFR1 output is connected to other signaling pathways activated by either lipid receptors (Lpa1) or G protein-mediated receptors (RasGRF1/2) (http://www.iib.uam.es/script/publicaciones.es.cgi?cod=258). To identify the main effectors of IGF-I in hearing, the analysis of auditory brainstem responses (ABR) and cochlear morphology of mutant mice deficient in the above mentioned signaling molecules was carried out and showed that both IRS2 and c-RAF are essential for cochlear development and hearing. In parallel, novel IGF-I targets in the mouse cochlea were identified by comparing *Igf1*$^{-/-}$ and *Igf1*$^{+/+}$ transcriptomes using RNA microchips. IGF-I deficit caused a delayed maturation of neural cells that associated with alterations in the levels and expression patterns of the transcription factors *Six6*, *Mash1* and of the myocyte enhancing factor 2 (MEF2) in the cochlea. IGF-I deficit was compensated, at least in part, by the up-regulation of the forkhead box M1 (FoxM1) transcription factor (http://www.ncbi.nlm.nih.gov/pubmed/20111592?otool=iescsiclib).

In conclusion, we have defined the spatiotemporal expression of elements involved in IGF signalling during inner ear development and reveal novel regulatory mechanisms that are modulated by IGF-I in promoting sensory cell and neural survival and differentiation. A combination of mouse genetics with non-invasive hearing evaluation screening has provided further insight into the molecular basis of IGF-I actions These data will help us to understand the molecular bases of human sensorineural deafness associated to deficits in IGF-I.

Challenges of the genetic diagnosis of highly heterogeneous disorders

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Human retinal dystrophies (RD) are a group of hereditary visual disorders characterised by extreme genetic and clinical heterogeneity. Genetic testing of this group of diseases is of outmost importance as human gene-specific clinical trials to improve photoreceptor recovery and survival are emerging. Although the knowledge of the molecular bases of visual disorders has greatly increased during the last years, particularly of retinal dystrophies, a comprehensive clinical and genetic diagnosis is still absent. The high number of pathogenic genes involved and the fact that there are no prevalent mutations constitute the main drawbakcs to standarized protocols. High-throughput technologies have provided rapid, cost-effective and reliable assays to perform direct or indirect screening of putative pathogenic variants. Direct screen for reported mutations rely on commercial disease chips (Asper Ophthalmics) and microarray-based sequencing methods (Affymetrix).

We have combined high-throughput SNP-based genotyping with conventional cosegregation analysis to diagnose familial cases of autosomal dominant and recessive RP and Leber Congenital Amaurosis (LCA) (Pomares et al., 2010). The chip has been designed to discard candidate genes in a single genotyping step, underscoring those to be screened for mutations. The effectiveness of this chip depends on the pedigree size and structure and genetic informativity. Large families as well as recessive consanguineous pedigrees are highly informative and, in most cases, allow the exclusion of all genes but one, directly highlighting the candidate for further study. Moreover, considering that exclusion of all candidates is a pre-requisite to undertake gene search, our approach provides an unvaluable tool to single out families suitable for genome-wide linkage analysis.

The identification of the genes and mutations causing retinal disorders is of utmost importance to understand the pathways that regulate photoreceptor survival, improve the diagnostic accuracy and unveil effective therapeutical targets.

Pomares E, Riera M, Permanyer J, Méndez P, Castro-Navarro J, Andrés-Gutiérrez A, Marfany G, Gonzàlez-Duarte R. Eur J Hum Genet. 18:118-24, 2010.

RetChip1.0 – A novel array-based tool for diagnostic testing in hereditary retinal degenerations

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A hallmark of the hereditary retinal degenerations is their striking genetic heterogeneity, i.e. a single clinical phenotype may be caused by mutations in many different genes and an even greater number of gene-specific mutations. Vice versa, mutations in a single gene may cause strikingly diverse clinical phenotypes. This makes genetic testing in hereditary retinopathies time-, labour- and thus highly cost-intensive. Despite these obstacles, the availability of genetic diagnosis in retinal dystrophies is an essential part of clinical diagnosis and is indispensable in patient management and to clarify individual and recurrent risks.

Addressing this situation, the array based re-sequencing technology may offer a cost-efficient high-throughput solution. The simultaneous analysis of a large number of disease genes provides a capacity to re-sequence up to 300,000 bases of double-stranded DNA on a single array. RetChip1.0 includes 72 genes hitherto associated with generalized RP (excluding stationary RP), Stargardt disease, Bardet-Biedl syndrome and Usher syndrome. The 72 genes comprise 1147 corresponding coding exons which need to be individually amplified prior to chip analysis. We have established a multiplex strategy which reduces the number of PCR reactions by approximately 75%. The evaluation of detected sequence alterations is performed with a software tool developed in cooperation with JSI-medical systems (Kippenheim, Germany) and offers the necessary high degree of automation with implemented quality control.

The genetic testing with RetChip1.0 is feasible in 7 modules (RP simplex, adRR, arRP, LCA, CRD, Bardet-Biedl syndrome, Usher syndrome) and is done in selected centres of excellence in Germany where clinicians, genetic counsellors and molecular geneticists work closely together. The testing is accompanied by a comprehensive statistical/biometrical evaluation of the data. Together this setting will ensure the development of standardized structures for DNA testing and will facilitate the assessment of pros and cons in the implementation of this novel and complex technology.

The Otochip sequencing array for hearing loss and Usher syndrome

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Despite the identification of many genes for pediatric hearing loss, few clinical tests have been developed, largely due to the high cost of sequencing. To address this, we have developed the OtoChip™, an oligo-hybridization array which sequences 19 genes involved in nonsyndromic and syndromic hearing loss. The genes sequenced by the OtoChip™ include: *GJB2* (except 35delG), *GJB6, MTRNR1, MTTS1, PDS, OTOF, MYO6, TMPRSS3, TMIE, TMC1, MYO7A, USH1C, CDH23, PCDH15, USH1G, USH2A, DFNB31, GPR98* (partial), and *CLRN1*. This test is recommended for screening patients with apparent nonsyndromic sensorineural hearing loss (NSSNHL) who have tested negative for DFNB1 mutations as well as patients with possible Usher syndrome. The test uses 430 PCR reactions multiplexed into 92 pools and detects greater than 97% of substitutions, 95% of known insertions and deletion (indels), and 37% of novel indels. The known indels are detected using tiled genotyping probes for 280 previously reported or detected indels in these 19 genes. We analyzed the first 50 OtoChip cases performed in our clinical lab (Laboratory for Molecular Medicine, Partners Center for Personalized Genetic Medicine). Six were excluded from sensitivity analyses because the patients had phenotypes that were inconsistent with the recommended indication (unilateral hearing loss-3, conductive hearing loss-2 or temporal bone abnormalities-1). The majority of individuals (61.4%) were Caucasian with 6.8% Black, 4.6% Hispanic, 4.6% Ashkenazi Jewish, 9.1% multi-racial, and 13.6% having an unspecified ethnicity. Of the 44 analyzed cases, 32 had NSSNHL and 12 had Usher syndrome. Over 200 rare mutations were identified, subjected to variant assessment (literature review, computational analysis, population frequency, etc) and classified with a conservative assignment of clinical significance. This analysis led to the following clinical reports: 7 (16%) positive, 15 (34%) inconclusive (due to absence of variants in both alleles and/or variants of unknown significance) and 22 (50%) negative (see Table 1). The variants identified in positive cases are listed in Table 2. Of the 7 clearly positive cases, 4 had no evidence of Usher syndrome (tested at 0.5, 1, 3, 5 yrs) but have mutations in genes associated with a high (cases 1-3) or moderate (case 4) likelihood of developing retinitis pigmentosa consistent with Usher syndrome. In summary, the OtoChip represents a useful clinical test to identify an etiology of Usher syndrome or hearing loss (in DFNB1 negative patients) including early diagnosis of Usher syndrome prior to the onset of retinitis pigmentosa.

Table 1. Sensitivity by phenotype

	Positive	**Inconcl.**	**Negative**	**Total**
NSSNHL	4 (13%)	12 (25%)	16 (50%)	**32**
Usher	3 (25%)	3 (25%)	6 (50%)	**12**
Total	**7 (16%)**	**15 (34%)**	**22 (50%)**	**44**

Table 2. Variants identified in positive cases

Patient (Result)	**Allele 1**	**Allele 2**
1-NSSNHL	E166fs-*MYO7A*	H1109fs-*MYO7A*
2-NSSNHL	C652fs-*MYO7A*	C652fs-*MYO7A*
3-NSSNHL	C1447fs-*USH2A*	P2811T -*USH2A*
4-NSSNHL	R1746Q-*CDH23*	D2148N-*CDH23*
5-Usher	R147H-*MYO7A*	A1540V-*MYO7A*
6-Usher	S211G-*MYO7A*	Q1178P-*MYO7A*
7-Usher	Q2138fs-*CDH23*	Partial gene deletion

Asper's diagnostic tool for Usher syndrome

Maigi Külm and Ilona Lind

Asper Biotech offers convenient Usher syndrome genotyping microarray for testing mutations in 9 genes associated with Usher syndrome.

The microarray is designed with the arrayed primer extension (APEX) technology (Kurg et al, 2000), which successfully detects single nucleotide changes, deletions and insertions in heterozygous and homozygous patient samples (Tonisson et al, 2000, 2002). In short, 5´-modified sequence-specific oligonucleotides, designed with their 3´end immediately adjacent to the variable site, are arrayed on a glass slide. PCR-prepared and fragmented target DNA is hybridized to oligonucleotides on the slides, followed by sequence-specific extension of the 3´ends of primers with dye-labelled nucleotide analogs (ddNTPs) by DNA polymerase. The covalent bonds between an oligonucleotide and a dye terminator are subsequently detected and analyzed. The time required for complete analysis, including sample preparation, is less than 4 hr.

The main disadvantage of the USH array is that it detects only previously known mutations. This can be compensated by the flexibility of the method - the new mutations are added to the microarray each year. Current version of Usher genotyping microarray detects 596 pathologic variants in nine genes compared to the first version´s 298 pathologic variants in eight genes. The other disadvantage is that sequencing confirmation is required for the found mutation(s).

The efficiency of the microarray was analysed using DNAs from 370 unrelated Usher syndrome patients from the Europe and USA revealing sequence variants in 46% of patients with Usher syndrome type I, 24% of patients with Usher syndrome type II, 29% of patients with Usher syndrome type III and 30% of patients with atypical Usher syndrome (Cremers FPM et al, 2007).

The genotyping microarray is a low-cost and rapid technique, which can be used as the initial mutation screening tool for patients with Usher syndrome.

New strategies/technologies to identify new genes

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In the past, the identification of recessive disease genes was mainly based on linkage analyses in large consanguineous families and subsequent positional cloning of the respective disease genes. However, large families suitable for linkage studies are rare, and many have already been characterized genetically. New strategies circumvent this limitation by homozygosity mapping in small families, looking for homozygous regions shared by affected members of several consanguineous families (each with several potentially linked regions), taking advantage of high resolution SNP arrays.

In addition, the detailed characterization of the Usher protein complex provides the basis for candidate approaches aimed at the identification of new disease genes. We have recently proven the value of this strategy, combined with linkage techniques, by the identification of *DFNB31* as the USH2D gene. Large deletions have been described in the Usher genes *PCDH15* (USH1F) and *GPR98* (USH2C). High-resolution comparative genomic hybridization (array-CGH) can therefore not only identify causative structural alterations in Usher genes, but may also provide the initial indication for novel Usher loci.

The introduction of the zebrafish as a model organism for human disease has several implications for the research on Usher syndrome: One major advantage is the morpholino knockdown technique. Microinjection of early embryos with morpholino oligonucleotides results in transient gene knockdown, enabling researchers to characterize gene-specific developmental phenotypes and to rapidly assess candidate genes for Usher syndrome. In contrast to most mouse models, zebrafish models for Usher syndrome display a retinal phenotype, providing a valuable source for the study of this sensory deficit.

Finally, the current revolution in sequencing technology has already changed approaches for disease gene identification and diagnostics. Massively parallel sequencing has become feasible and will soon replace gene-by-gene positional cloning strategies. It will also uncover Non-Mendelian genetic constellations such as the influence of modifiers.

The Usher protein network in the inner ear

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Nine of the genes involved in Usher syndrome have been identified; *MYO7A, USH1C, CDH23, PCDH15, USH1G, USH2A, GPR98, DFNB31,* and *USH3A.* They encode proteins that belong to very different functional categories such as adhesion proteins (cadherins), signaling proteins (G-protein coupled receptor), and scaffold proteins. For all but one of these genes, *MYO7A,* alternative splicing has been shown to result inseveral different protein isoforms.
Mice with defects in Usher genes already indicated a role for these proteins in the development, maintenance and function of the stereocilia bundle of inner and outer hair cells. The mutants exhibit defects in length, width, cohesion and number of stereocilia and also the orientation of the hair bundle which is determined by the planar cell polarity pathway. The co-functioning of the Usher proteins in a complex with the scaffold proteins anchoring the transmembrane proteins with large extracellular regions to the actin core of the stereocilia explains the inner ear abnormalities in the mouse mutants and hearing loss in humans at least in part and it is now known that the stereociliar links including the tiplink and also kinociliar links are composed of and anchored by Usher proteins in a specific spatiotemporal pattern. In addition, whirlin (*DFNB31*) is known to be involved in the organization of protein complexes that are responsible for stereocilia elongation. The role of clarin1 (*USH3A*) in the hair bundle is less well understood. Also the specific functions of different isoforms of Usher proteins are largely unresolved but very recent studies in mouse models begin to demonstrate that there are tonotopic diffences in their localization.

Besides in the apical part of hair cells, several of the Usher proteins are localized in the synaptic region of these cells, but the exact localization and function in either hair cells, or afferent or efferent nerve terminals remains to be elucidated. There are indications for a role in the organization of ion channels in the synapses. However, so far there are no clear indications for structural or functional abnormalities of the synapses of inner and outer hair cells in mice with mutations in Usher genes.

The usher protein network in the retina

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Usher syndrome (USH) is the most frequent cause of combined deaf-blindness in man. It is clinically and genetically heterogeneous and at least twelve chromosomal loci are assigned to three clinical USH types. Although the three USH types exhibit similar phenotypes in human, the corresponding gene products of the nine identified genes belong to very different protein classes and families.

Molecular analysis of USH protein function revealed that all USH1 and USH2 proteins are integrated into protein networks via directly binding to the scaffold proteins harmonin (USH1C), SANS (USH1G) and whirlin (USH2D). In the inner ear, these interactions are essential for the differentiation of hair cell stereocilia but may also participate in the mechano-electrical signal transduction and the synaptic function of maturated hair cells.

The late on set of the USH disease in the retina indicates dysfunction of retinal USH protein networks in the mature retina. The co-expression of all USH1/2 proteins at the synapses in the outer plexiform layer indicates a functional role of USH protein networks in retinal synapses. In addition, we and others described a subset of components of the USH protein interactome in the ciliary region of photoreceptor cells. There is growing evidence that this periciliary USH protein complex is closely related to the intracellular and ciliary transport by integrating the molecular machinery for the cargo transfer from the inner segment transport to the transport module for ciliary delivery. Moreover, our recent studies on primate photoreceptors indicate a role of USH proteins in the function of calycal processes.

In conclusion, decipherment of the composition USH protein interactome defines the role of USH proteins in photoreceptor cell biology and thereby elucidates potential targets for treatments and cure of the disease.

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