

Friday 4th of July, 2008

Key note lecture

Retinal dystrophy research: The past, the present and the future

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Fifty years ago little was known about retinal dystrophies, save that it was genetically determined and a small number of individual disorders had been recognised on the basis of their distinctive phenotypes. The first clue as to the complex nature of RP was the demonstration that in some families visual loss occurred because of cell death and in some it is due to cell dysfunction. This was followed by finding the genes responsible for disease, identification of the respective proteins and their function.

Three biological approaches to treatment of retinal dystrophies have been developed in the last few years, namely the use of growth factors, gene therapy and cell transplantation. It is important for the clinician to prepare for the introduction of treatment into clinical practice, so that the potential benefits of scientific research can be realised

Clinical setting for treatment:

It is evident that a large well-documented patient pool is necessary to realise the full benefits of treatment, should this be introduced into clinical practice. Certain attributes of disease had to be established prior to initiating therapeutic studies.

- 1. Identification of the causative genes.* Currently it should be possible to identify the responsible gene in about 50% of families with autosomal dominant disease, and most if not all X-linked disease. For autosomal recessive disease the number is uncertain. To achieve this objective genotyping laboratories are required.
- 2. Knowledge of the disease mechanisms.* Whether disease is due to haplo-insufficiency or gain of function will determine a suitable approach to gene therapy. Equally important is the identification of the cell that expresses the mutant gene.
- 3. Temporal profile of functional loss and cell death.* It has been shown that in some disorders cell dysfunction may precede cell death by several years and in others loss of function is due to cell death. In the first case gene therapy may cause recovery of function, and cell transplantation would be unsuitable. In the second cell transplantation would be appropriate and gene therapy may slow the degenerative process.

4. Detection of the therapeutic effect: The techniques of recording the treatment effect must be available such as electroretinography, psychophysics and specialised imaging.

To-date therapeutic trials have been initiated for growth factors in advanced RP and gene therapy for a rare form of Leber amaurosis. It is very early to draw any definite conclusions as to their efficacy but there is clearly promise. It is hope that these forms of treatment will be proven and extended such that an impact on blindness is achieved.

Notes.

Science block 1: (English only, no interpretation)
Clinical diagnosis of Retinal Dystrophies (RD)

Clinical diagnosis of RD and its value for the Swedish RP registry

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The Swedish RP Registry is linked to the Department of Ophthalmology at the University of Lund. It comprises over 2,700 patients including pedigrees from patients examined at the unit in Lund with electrophysiology and a thorough ophthalmological investigation. As the Committee of Ethics also has approved obtaining DNA samples in a BioBank numerous molecular studies have been completed and the gene defects in more than 250 families with different mutations in 30 genes have been identified.

Full field electroretinography is the method of choice for objective evaluation of the total retinal function, and has been used widely in experimental and clinical research for characterization of retinal degenerations. During recent years new electrophysiological techniques, have enhanced our possibilities to better investigate and understand the function in separate areas of the retina and the visual pathway. The department of Ophthalmology in Lund was the first clinical unit in Scandinavia, to use multifocal ERG for further studies of localized dysfunction in the retina. A further development of multifocal ERG is multifocal VEP, which reflects the cortical response to a localised stimulation of the eye. Multifocal VEP continuously improves, and seems to be of most important value for an objective way to further investigate the visual pathway.

Recent development of clinical electrophysiology, molecular genetics has led to a radical improvement in understanding etiology and pathophysiology of hereditary retinal disorders.

Syndromic RP

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Syndromic Retinitis Pigmentosa comprises nearly 200 conditions. Several Finnish colleagues: Forsius, Karjalainen, Kivitie-Kallio, Norio, Raitta, Santavuori and

Sankila have contributed to a better understanding of the disorders Usher syndrome, Ceroid lipofuscinosis and Cohen syndrome. The clinical diagnostics are based on a good family history and an examination of the whole patient including stature and intellectual presentation. In addition to a thorough eye examination a description of any dysmorphic findings of the hair, face, skin, teeth or skeleton can give important handles for diagnosis. Fortunately it is not necessary to remember the names of all rare syndromes since we to-day have an important diagnostic instrument in the database "Geneeye". Cooperation with other specialists is essential, for example might a hearing test lead to the diagnosis of Usher syndrome. The clinical classification has been based on both aetiologies like metabolic diseases and symptoms like neurological disorders. The genetic classification is still improving, now with 48 genes for syndromic retinal diseases identified. However, many of these disorders are heterogeneous with both genetic and clinical variation. It is therefore a great help in daily clinic that some of these syndromes now can be diagnosed by genetests on commercial basis. From a clinical point of view just a few systemic RP disorders have a specific therapy. One example is Refsum disease where RP is combined with certain neurological symptoms. Refsum disease is caused by deficiency of the peroxisomal enzyme phytanic acid 2-hydroxylase. The accumulation in blood and tissues of phytanic acid cause cell damage, but the concentration can be lowered by restriction of certain milk and meat products. This has been shown to reduce the rate of further deterioration of primarily the neuropathy and possibly the retinal degeneration.

During recent years new progress in classification and further insights in several aspects of mammalian development and function of organs were developed by the study of Bardet-Biedl syndrome (BBS) characterized by RP, obesity, cognitive impairment, skeletal and teeth anomalies and genito-renal abnormalities. There are at least 12 involved genes, where the BBS10 gene with the mutation C91fsX95 now seems to be important. There is no obvious phenotype-genotype relationship in BBS, it has therefore been hypothesised that the different BBS proteins are functioning through a common final pathway.

It had been noted that patients with BBS8 mutation often had situs inversus, a condition in which normal left-right symmetry of the internal organs is partly disrupted. In the third postfertilisation week a faint elevation on the flat embryo is produced by the primitive node and streak. Ciliated cells in the primitive node establish a signal that is essential for normal left-right asymmetry. It could be shown in animal models, that lack of ciliary function could be compatible with both RP, renal and lung disorder and defective function of genitalia. Also reduced hearing and anosmia could be explained by ciliary dysfunction. Cilia are small organelles that projects from the surface of most cells and can be both motile and immobile. Other RP ciliopathies include Alström syndrome, Leber congenital amaurosis, Usher syndrome, Senior-Løken syndrome and Joubert syndrome.

New promising projects are also the studies of “Genetic obesity syndromes” including the RP syndromes BBS, Alström and Cohen syndromes. In mouse models of BBS2, BBS4 and BBS6 disturbances in Leptin regulation have been reported. Leptin is a hormone involved in appetite and energy homeostasis. The genetic causes and functional disturbances of these genetic obesity syndromes could gain tremendous insight into the pathways to obesity.

Syndromic RP disorders therefore are in focus not only because of the individual but also of the general implications their study may involve.

Early onset RP (LCA)

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Early-onset retinal dystrophies refer to a genetically heterogeneous group of disorders that are usually of autosomal recessive inheritance. Leber congenital amaurosis (LCA) is a common causes of hereditary blindness that manifests in infants or in the first few months of life. LCA is typically characterized by nystagmus, sluggish or no pupillary response, severe and progressive vision loss in an otherwise healthy child. It is one of the most common genetic causes of congenital visual impairment in infants and children.

To date, mutations in 13 known loci have been implicated in LCA causation. An examination of the known LCA genes highlights several underlying pathological processes including photoreceptor development and maintenance, phototransduction, vitamin A metabolism, and protein trafficking among others. It has been known for some time that defects in sensory cilia can cause photoreceptor cell death and more recently a subset of LCA can also be referred to as a "ciliopathy."

Genes implicated in LCA causation include retinal guanylate cyclase (*GUCY2D*) on chromosome 17p13.1 (LCA1), *RPE65* on chromosome 1p31 (LCA2), *RDH12* on chromosome 14q23.3. to be clarified), *AIP1* on chromosome 17p13.1 (LCA4), *RPGRIP1* on chromosome 14q11 (LCA6), *CRX* on chromosome 19q13.3 (LCA7), *CRB1* on chromosome 1q31.3 (LCA8), *CEP290* on chromosome 12q21.3 (LCA10), and *IMPDH1* on chromosome 7q32.1 (LCA11). Mutations in *LRAT* (4q32.1) and *TULP1* (6p21.31) genes have also been identified in recessive LCA. LCA9 has been mapped to 1p36, but a causative gene has not been identified. The location of the LCA5 gene was mapped to 6q11-q16 and recently identified as *lebercilin*, previously known as C6orf152.

The role of the different genes and the range of related clinical phenotypes will be discussed.

Best and Stargardt disease

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Best macular dystrophy is an autosomal dominant macular degeneration caused by mutations in the *VMD2* gene. This gene encodes bestrophin, an integral membrane protein expressed in the retinal pigment epithelium (RPE). Bestrophin forms a chloride channel responsible for maintaining chloride conductance across the basolateral membrane of the RPE. The bestrophin deficit presumably underlies the retina-wide abnormality in the standing potential of the eye that is diagnostic of Best macular dystrophy prior to visible macular abnormalities. The characteristic appearance of prominent yellow macular lesions confirms a diagnosis of Best macular dystrophy during the evolution of the disease.

Stargardt disease is by far the most common form of macular degeneration to affect juveniles and young adults. There are two distinct forms, a rare autosomal dominant form caused by mutations in the *ELOVA4* gene, and a much more common autosomal recessive form caused by mutations in the *ABCA4* gene. Stargardt disease is characterized by discrete yellowish deposits within the posterior pole. The disease usually begins in the first or second decade of life, with visual acuity that cannot be corrected to 20/20. Typically, there is a fairly rapid decline in acuity during the teenage years, with final acuity of 20/200 – 20/400 by adulthood. The full-field ERG is useful in patients with *ABCA4* mutations for distinguishing the more common macular forms of dystrophy from the more widespread forms of cone-rod dystrophy. The multifocal ERG is particularly useful in patients with Stargardt macular dystrophy since it simultaneously measures retinal function at dozens of locations throughout the macula. In conjunction with spectral domain optical coherence tomography (SD-OCT), we have the necessary techniques in place for monitoring efficacy in clinical trials.

Several therapeutic paths are being simultaneously explored for patients with Stargardt disease. Planned gene therapy trials will attempt to transfect the retina with a normal copy of the *ABCA4* gene. This surgical approach has been used safely in recent gene therapy trials for Leber Congenital Amaurosis. Another approach to Stargardt macular dystrophy derives from knowledge of the function

of the *ABCA4* gene protein. The ABCR (*ABCA4*) protein is thought to play a crucial role in the recycling of products of the visual cycle in rods. Specifically, work with animal models has shown that *abcr* deficient mice build up toxic levels of photoreceptor waste in the RPE. One line of therapy, therefore, utilizes drugs known as *visual cycle modulators* to slow down rod activity, especially in the daytime, in the hopes that the lowered metabolic load will protect the retina and lead to long-term survival of the cones.

Notes.

Patient block 1: Genetics (English-Finnish interpretation provided)

Genetics and Molecular Diagnostics of RD using DNA chips:
Knowing Our Genes and Mutations

Andreas Gal, Professor

Institute of Human Genetics, University Medical Centre Hamburg-Eppendorf

Human retinal dystrophies show an unparalleled diversity. Genetically, a disease phenotype in a family may follow any of the Mendelian patterns of inheritance (autosomal dominant, autosomal recessive, and X-chromosomal). In addition, mitochondrial transmission, digenic (diallelic or triallelic), and complex genetic mechanisms (e.g. uniparental disomy) have also been described. Clinically, ophthalmological symptoms and signs due to genetically distinct entities may be largely overlapping. At the same time, patients from the same family or those being apparently unrelated but carrying the same mutation may present with very different clinical pictures.

As of May 2008, RetNet, a genetic database specialized for human retinal diseases, lists 26 different clinical entities caused by mutations of at least 193 genes: 142 already known and 51 whose identity is not yet defined. In particular, mutations of 30 and 9 known genes may cause RP and Usher syndrome, respectively, whereas 12 genes implicated in these two conditions have established chromosomal locations, but their identity is still unknown. According to the function of the encoded proteins, the 39 RP and Usher genes can be assigned to a number of different groups, such as structural proteins of the photoreceptors, proteins involved in the phototransduction cascade, visual cycle (vitamin A metabolism), or intracellular transport processes.

Identification of the individual genetic defect is becoming more and more important for every patient for it may provide confirmation of a precise diagnosis, some information on course and prognosis of the disease, and, especially, and define the pattern of inheritance including the disease risk of family members. Knowing the genetic defect is also a prerequisite to devise targeted forms of therapy.

However, the immediate use of the results provided by the genetic research for patient care has been hampered both by the large number of disease genes and the extensive sequence variation occurring in the majority of the retinal dystrophy genes. Many of the disease mutations are unique ('private mutations'), i.e. they occur in a single family or in a small number of families.

While this diversity caused serious obstacles in terms of finances, turn-around time and labor intensity in the past, these problems can now be overcome in part by high throughput (array/chip) technology developed during the past decade. The mutation detection chip is designed to pick up sequence variants that have been identified in (other) patients previously. Chips contain gene mutations according to

the disease phenotype expected and the pattern of inheritance. Since these two variants cannot always be defined with certainty, multiple analyses may be necessary. Although this type of array has a number of limitations, this approach offers an excellent first pass opportunity of genotyping patients in a rapid way at affordable costs. The sequencing chip scans a large number of genes and is able to detect both known and not yet reported mutations. Pro Retina Germany is supporting a consortium that brings together geneticists and ophthalmologists to develop such a sequencing chip for routine diagnostics. RetChip 1.0 was designed by Prof. Weber and his group (University of Regensburg) and should provide detailed information on individual sequence variants of a total of 72 genes implicated in the pathogenesis of various forms of RP, Leber congenital amaurosis (LCA), macular dystrophy, Usher syndrome, and Bardet Biedl syndrome.

Genetic Counseling and Gene Tests

Helena Kääriäinen, Research Professor
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Genetic counseling is a communication process that deals with the occurrence, or risk of occurrence, of a (possibly) genetic disorder in the family. The process involves an attempt by appropriately trained person(s) to help the individual or the family to (1) understand the medical facts of the disorder; (2) appreciate how heredity contributes to the disorder and the risk of recurrence in specified relatives; (3) understand the options for dealing with the risk of recurrence; (4) use this genetic information in a personally meaningful way that promotes health, minimizes psychological distress and increases personal control; (5) choose the course of action which seems appropriate to them in the view of their risk and their family goals, and act in accordance with that decision; and (6) make the best possible adjustment to the disorder in an affected family member and/or to the risk of recurrence of that disorder (modified from Frazer FC: Genetic counseling. *Am J Hum Genet* 1974;26:636-661, Biesecker and Peters: Process Studies in Genetic counseling: peering into the black box. *Am J Med Genetics* 2001;106:191-198). In real situations the content of genetic counseling varies according to the wishes of the counselees: some want detailed medical data while others need support to their life choices.

In case of hereditary retinal disorders, genetic counseling should always be offered to patients/family members who can then themselves decide whether to accept the offer or not.

In case of rare diseases, genetic counseling often takes place as part of a simultaneous diagnostic consultation.

Genetic tests are performed for different purposes. In retinal disorders, genetic testing may be part of the diagnostic work up in a symptomatic individual to find out the exact reason for the visual problem. As the clinical findings may be undistinguishable in different types of retinitis pigmentosa with different modes of inheritance, finding the mutation(s) may be the only way to clarify the diagnosis. However, a genetic test is not always possible and thus the diagnosis (and genetic counseling) is very often based on clinical findings.

Sometimes, however, gene tests are used for other purposes like detecting a carrier, predicting a future disease or making a prenatal diagnosis. These tests may have profound implications for the individual and the relatives as well. In relation to such tests, appropriate genetic counseling by a professional in the field of genetics should always precede testing. In addition, the implications of the test to the individual and his/her family should be explained in a post-test genetic counseling session. This is clearly written to the recommendations of outstanding international organizations, like Unesco, OECD and Council of Europe, as well as of professional groups or organizations like EuroGentest and European Society of Human Genetics.

Gene therapy of USH 3

John Flannery, Professor

Helen Wills Neuroscience Institute, Berkeley, California, USA

Major progress in understanding the molecular causes of Usher syndrome has been made in the last few years. Usher syndrome was initially categorized by its clinical appearance as two distinct types of autosomal recessive deaf-blindness: Usher I patients had profound congenital sensorineural deafness, vestibular dysfunction and retinitis pigmentosa (RP). Usher type II patients had moderate congenital hearing impairment, normal vestibular function, and RP. A third USH subtype was later identified as clinical ophthalmologists noted progressive hearing loss in some patients previously diagnosed with RP. At the same time, molecular geneticists identified a gene defect in (Clarín-1, CLRN1), a causative gene distinct from the other Usher subtypes. Usher type 3 is now universally accepted as a separate disease subtype characterized by progressive (non congenital) hearing loss, variable vestibular abnormality and RP. Usher 3a is relatively rare in most parts of the world; however, it represents about 40% of Usher patients in Finland and in Ashkenazi Jews in Europe and North America.

The structure of the clarín-1 gene (CLRN1) is now known and its coding sequence

comprises 699 base pairs. The protein encoded by the gene is a tetraspanin, with a hypothesized function in sorting and trafficking proteins to the rod photoreceptor outer segment through the connecting cilium. The small size of the clarin coding sequence makes it a highly attractive candidate for gene therapy because it may be delivered using the recombinant Adeno associated virus (AAV) technology. The safety and efficacy of using AAV vectors for gene therapy for recessive retinal degeneration has recently achieved proof of concept in the three clinical trials for Leber's Congenital Amaurosis, an autosomal recessive retinal degeneration. Usher 3 is also an autosomal recessive disease and one potential treatment will be to deliver a normal copy of the mutated clarin -1 gene to the photoreceptor cells using AAV vectors. Usher 3a is the slowest progressing of the three types of Usher syndrome, which increases the time window into which a gene replacement therapy may be applied.

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Science block 2: Genetic diagnosis and research (English only, no interpretation)

Genetics and Molecular Diagnostics of RD using DNA chips:
Knowing Our Genes and Mutations

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Human retinal dystrophies show an unparalleled diversity. Genetically, a disease phenotype in a family may follow any of the Mendelian patterns of inheritance (autosomal dominant, autosomal recessive, and X-chromosomal). In addition, mitochondrial transmission, digenic (diallelic or triallelic), and complex genetic mechanisms (e.g. uniparental disomy) have also been described. Clinically, ophthalmological symptoms and signs due to genetically distinct entities may be largely overlapping. At the same time, patients from the same family or those being apparently unrelated but carrying the same mutation may present with very different clinical pictures.

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identified in (other) patients previously. Chips contain gene mutations according to the disease phenotype expected and the pattern of inheritance. Since these two variants cannot always be defined with certainty, multiple analyses may be necessary. Although this type of array has a number of limitations, this approach offers an excellent first pass opportunity of genotyping patients in a rapid way at affordable costs. The sequencing chip scans a large number of genes and is able to detect both known and not yet reported mutations. Pro Retina Germany is supporting a consortium that brings together geneticists and ophthalmologists to develop such a sequencing chip for routine diagnostics. RetChip 1.0 was designed by Prof. Weber and his group (University of Regensburg) and should provide detailed information on individual sequence variants of a total of 72 genes implicated in the pathogenesis of various forms of RP, Leber congenital amaurosis (LCA), macular dystrophy, Usher syndrome, and Bardet Biedl syndrome.

Genetic counseling in Finland

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Genetic counseling in Finland was performed from 1952 in a small scale at Väestöliitto, a state funded union of associations dealing with family, population and social affairs. This "Genetics Board" was consulted at the time, when permission for the termination of pregnancy needed special grounds like a risk for an inherited disease. Every now and then, in addition to the official decision, the parents were counseled as well. At the same time, the diseases of the "Finnish Disease Heritage" (Norio R, 2003) began to emerge, and need for a proper genetic counseling clinic became evident. This led to the foundation of first official national centre for genetic counseling, Department of Medical Genetics of Väestöliitto in 1971 (http://www.vaestoliitto.fi/in_english/genetics/). Genetic counseling was offered for the whole country in all fields of medicine in co-operation with respective specialists. A unit for prenatal diagnostics was established at the Department of Obstetrics and Gynaecology at Helsinki University Hospital in 1977. Samples of amniotic fluid were sent by air to the Laboratory of Prenatal Genetics from three other university hospitals.

The long distances in a rather sparsely populated country outside the capital area of Helsinki lead in the beginning of the 1980's to a second medical genetics unit with laboratory and prenatal diagnostics services. It was founded at Oulu University Hospital in northern Finland. Today there are eight genetic counseling clinics for the five million Finns. The remaining three University Hospitals in Kuopio, Tampere and Turku have got their Departments of Medical Genetics one

by one. To all of them patients wishing for genetic counseling can be referred to. Some of the clinics for other specialities have their own genetic out patient clinics. One of these is the out patient clinic for genetic eye diseases which was founded in 2000 at the Department of Ophthalmology at Helsinki University Hospital. Even is genetic counseling is not offered there as such, it co-operates efficiently with the genetic counseling clinics in the country. There are two private but state funded clinics in Helsinki. The Department of Medical Genetics at Väestöliitto works now in the field rare diseases in general and genetic counseling of the rare dysmorphic syndromes in national level and Folkhälsan Department of Medical Genetics serves mainly the Swedish-speaking population of Finland.

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Gene Transfer and Expression of “photoswitches” Confers Light Sensitivity to Retinal Ganglion Cells

John Flannery, Kate Kolstad, Richard Kramer, Dirk Trauner, Ehud Isacoff, Kenneth Greenberg.

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Introduction: Retinal degenerations cause blindness by several mechanisms culminating in the death of photoreceptor cells. Pharmacological, gene replacement, and neuroprotective strategies attempt to postpone cell death in patients with surviving photoreceptors, however many individuals are not candidates as their photoreceptors have been lost. In many retinal degenerations, inner retinal neurons survive after photoreceptor apoptosis. One strategy for restoration of light perception is by conferring light sensitivity to inner neurons in the retina. Some vision may be restored if the remaining neurons are genetically engineered to directly respond to light and signal to visual cortex.

Methods:, Light-sensitive cation channels from bacteria (Channelrhodopsins) and anion pumps (Halorhodopsins) can excite and inhibit neural activity in response to light. Mammalian ion channels have been genetically engineered to add a light responsive function, specifically, shaker K⁺ - ('SPARK') for Synthetic

Photoisomerizable Azobenzene Regulated Potassium channel and a light-gated ionotropic glutamate receptor (LiGluR).

In addition to expression of “photoswitches” in retinal ganglion cells, restoration of light perception will require targeting these “photoswitches” appropriately, to create a virtual ON or OFF signaling pathway. The human retina contains 30 morphologically distinct bipolar and ganglion cell subtypes; with ON and OFF-center signal detection as the most significant division among visual features extracted by ganglion cells. Imparting light sensitivity specifically to ON or OFF-center bipolar and ganglion cells with excitatory or inhibitory photoswitches may allow light perception in the absence of rod and cone-mediated vision.

Results: We have targeted transgene expression in ON-type retinal ganglion cells in vivo. An AAV vector with a connexin-36 promoter will drive expression of the SPARK and LiGluR ion channels specifically in ON-type retinal ganglion cells in normal rat retina and the P23H rat model of rhodopsin RP. In vivo fundus imaging and confocal microscopy demonstrated GFP expression exclusively in ON-type retinal ganglion cells. The dendrites of transduced ganglion cells stratify in sublamina B of the IPL, adjacent to the ganglion cell bodies, where they make synaptic contact with the axon terminals of ON-type bipolar cells signaling increments of light.

Discussion: We conclude that specific expression of excitatory and inhibitory ‘photoswitch’ in ON-type and OFF-type retinal ganglion cells is achievable, and that firing activity of these cells can be modulated by light.

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Notes.

Patient block 3: Clinical aspects of RD (English-Finnish interpretation)

Diagnosis and treatment of concurrent eye diseases

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University of Helsinki

The most common concurrent eye diseases related to RP are posterior subcapsular cataract (PSC) and cystoid macular edema (CME). In addition, all patients develop vitreous changes. Peripheral exsudative vasculopathy (Coats' response) is a rarer coincidence.

PSC is common already in young and middle aged persons. The main symptoms are variable vision in different light and glare or bright-light intolerance. PSC is diagnosed by routine ophthalmological examination and surgery is the only treatment. The mean age at surgery is about 50 years. The technique of surgery (mainly phacoemulsification and intraocular lens (IOL) implantation) does not differ much from that for other cataracts. Development of postoperative posterior capsule opacification (PCO) is common. After surgery, visual acuity (VA) improves in most eyes, but glare may remain and the visual field (VF) does not improve.

CME may occur in 10-20% of patients. It may decrease VA. CME is diagnosed by ophthalmoscopy, biomicroscopy, fluorescein angiography or optical coherence tomography (OCT). Various treatments have been attempted; at present systemic or topical carbonic anhydrase inhibitors (acetazolamide or dorzolamide) and intravitreal triamcinolone seem to be most promising although the results vary.

Vitreous changes and abnormal peripheral teleangiectic vasculature are diagnosed in clinical examination. Vitreous changes do not need treatment.

Teleangiectatic vasculature may be treated with laser or cryocoagulation, in some eyes more extensive retinal surgery is needed.

In addition to these diseases, patients with RP may suffer from refractive errors and other eye diseases as anyone else. However, the negative coincidence of RP and diabetic retinopathy, as well as RP and rhegmatogenous retinal detachment, was noticed already in the 1980ies.

Best and Stargardt disease

David G. Birch, Ph.D., Retina Foundation of the Southwest
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Best macular dystrophy is an autosomal dominant macular degeneration caused by mutations in the VMD2 gene. This gene encodes bestrophin, an integral membrane protein expressed in the retinal pigment epithelium (RPE). Bestrophin

forms a chloride channel responsible for maintaining chloride conductance across the basolateral membrane of the RPE. The bestrophin deficit presumably underlies the retina-wide abnormality in the standing potential of the eye that is diagnostic of Best macular dystrophy prior to visible macular abnormalities. The characteristic appearance of prominent yellow macular lesions confirms a diagnosis of Best macular dystrophy during the evolution of the disease.

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Syndromic RP

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Syndromic Retinitis Pigmentosa comprises nearly 200 conditions. Several Finnish colleagues: Forsius, Karjalainen, Kivitie-Kallio, Norio, Raitta, Santavuori and Sankila have contributed to a better understanding of the disorders Usher syndrome, Ceroid lipofuscinosis and Cohen syndrome. The clinical diagnostics are based on a good family history and an examination of the whole patient including stature and intellectual presentation. In addition to a thorough eye examination a description of any dysmorphic findings of the hair, face, skin, teeth or skeleton can give important handles for diagnosis. Fortunately it is not necessary to remember the names of all rare syndromes since we to-day have an important diagnostic instrument in the database "Geneeye". Cooperation with other specialists is essential, for example might a hearing test lead to the diagnosis of Usher syndrome. The clinical classification has been based on both aetiologies like metabolic diseases and symptoms like neurological disorders. The genetic classification is still improving, now with 48 genes for syndromic retinal diseases identified. However, many of these disorders are heterogeneous with both genetic and clinical variation. It is therefore a great help in daily clinic that some of these syndromes now can be diagnosed by genetests on commercial basis. From a clinical point of view just a few systemic RP disorders have a specific therapy. One example is Refsum disease where RP is combined with certain neurological symptoms. Refsum disease is caused by deficiency of the peroxisomal enzyme phytanic acid 2-hydroxylase. The accumulation in blood and tissues of phytanic acid cause cell damage, but the concentration can be lowered by restriction of certain milk and meat products. This has been shown to reduce the rate of further deterioration of primarily the neuropathy and possibly the retinal degeneration.

During recent years new progress in classification and further insights in several aspects of mammalian development and function of organs were developed by the study of Bardet-Biedl syndrome (BBS) characterized by RP, obesity, cognitive impairment, skeletal and teeth anomalies and genito-renal abnormalities. There are at least 12 involved genes, where the BBS10 gene with the mutation C91fsX95 now seems to be important. There is no obvious phenotype-genotype relationship in BBS, it has therefore been hypothesised that the different BBS proteins are functioning through a common final pathway.

It had been noted that patients with BBS8 mutation often had situs inversus, a condition in which normal left-right symmetry of the internal organs is partly disrupted. In the third postfertilisation week a faint elevation on the flat embryo is

produced by the primitive node and streak. Ciliated cells in the primitive node establish a signal that is essential for normal left-right asymmetry. It could be shown in animal models, that lack of ciliary function could be compatible with both RP, renal and lung disorder and defective function of genitalia. Also reduced hearing and anosmia could be explained by ciliary dysfunction. Cilia are small organelles that projects from the surface of most cells and can be both motile and immobile. Other RP ciliopathies include Alström syndrome, Leber congenital amaurosis, Usher syndrome, Senior-Løken syndrome and Joubert syndrome. New promising projects are also the studies of “Genetic obesity syndromes” including the RP syndromes BBS, Alström and Cohen syndromes. In mouse models of BBS2, BBS4 and BBS6 disturbances in Leptin regulation have been reported. Leptin is a hormone involved in appetite and energy homeostasis. The genetic causes and functional disturbances of these genetic obesity syndromes could gain tremendous insight into the pathways to obesity. Syndromic RP disorders therefore are in focus not only because of the individual but also of the general implications their study may involve.

Notes.

Session for general public (Finnish only, no interpretation)

Age related macular degeneration

Development of age-related macular degeneration

Kai Kaarniranta MD., PhD, MSc

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The average life expectancy in developed nations is over 80 years. However, the quality of life is often significantly diminished by the effects of age-related degenerative diseases, including age-related macular degeneration (AMD), which is the leading cause of blindness in the elderly worldwide. AMD is characterized by a progressive loss of central vision attributable to degenerative and neovascular changes in the macula, a highly specialized region of the central retina responsible for fine and colour vision. Complex interactions of many genetic and environmental factors are involved in the development of AMD. The most recent World Health Organization (WHO) global eye disease survey conservatively indicate that 14 million persons are blind or severely visually impaired because of AMD. The disease has a tremendous impact on the physical and mental health of the geriatric population and their families and is becoming a major public health burden and amounts are all the time climbing.

AMD is a progressive disease of the retina that affects the macula in the visual axis of the eye. AMD is characterized by morphological and functional abnormalities of the macular retinal pigment epithelial (RPE) cells, Bruch's membrane, and choriocapillaris. Degeneration and cell death of RPE cells cause secondarily adverse effects on neural retina leading to visual loss, but pathogenesis of AMD is still poorly understood. AMD results in an inability to read, recognize faces, drive, or move freely. AMD is divided early and late, as well as atrophic and exudative degeneration categories. Intracellular lipofuscin and extracellular drusen protein deposits are associated with AMD findings. Choroidal neovascularization is observed as a diagnostic finding in exudative AMD.

Prevalence of AMD is rising as a consequence of increasing age. In the absence of effective treatment for AMD, the number of patients severely disabled by AMD is expected to increase in the next 20 years by more than 50%. The prevalence of early AMD in the age-category 65–74 years is 15%, in the age-category 75–84 years 25%, and in persons 85 years and older 30%. A reasonable overall estimate of the prevalence of late AMD in persons aged 65–74 years is 1%, increasing to 5% in persons aged 75–84 years, and 13% in persons 85 years and older.

Current therapy options

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Advanced age-related macular degeneration (AMD) is the leading cause of irreversible central visual loss among elderly in the Western countries. In Finland 60% of new visual impairment annually is derived from advanced AMD giving rise to 800 visually handicapped in 2006¹. 80-90% of central visual loss in advanced AMD is caused by the exudative neovascular type of the disease.

The treatment of choroidal neovascularization (CNV) in AMD has been very challenging. In 1982 laser photocoagulation came as the first available treatment option for neovascular AMD. Thereafter photodynamic therapy with verteporfin and most recently the development of vascular endothelial growth factor inhibitors (anti-VEGF agents) have increased the treatment possibilities in eyes with exudative AMD. Today most of the eyes with CNV due to AMD are eligible for evidence-based therapy.

Indications for laser photocoagulation and photodynamic therapy (PDT) are reduced after the introduction of antiangiogenic substances. However, laser photocoagulation is still indicated for large recurrent extrafoveal CNV with some fibrosis². Photodynamic therapy with verteporfin can be the primary treatment option for small subfoveal lesions with recent disease progression if the follow-up is not possible to schedule according to novel anti-VEGF therapies². The recommendation to combine intravitreal steroid triamcinolone to PDT remains unanswered until the final results from on-going controlled studies are available.

Vascular endothelial growth factor inhibitors have recently changed the treatment culture of exudative AMD. Pegaptanib seems to have comparable treatment effect to photodynamic therapy regardless of lesion type or size². Ranibizumab will maintain the visual acuity in more than 90% of eyes with 30-40% of eyes gaining some vision after the treatment period of two years³. According to uncontrolled case series bevacizumab seems to have reasonable effect for exudative AMD but the final results of controlled studies are missing. In practice the need for repeated treatment sessions is a clear drawback in all of these anti-VEGF agents. Moreover, the long-term therapy with pegaptanib and ranibizumab comes remarkably costly.

In order to reduce the need for retreatment and to intensify the visual benefit combination therapies with anti-VEGF agents and PDT are going on. So far the

visual result seems to be unchanged but fewer treatment sessions can come possible.

Treatment strategies are rapidly changing. The neovascular lesion can be eligible for various treatment options. When considering the most appropriate therapy for the patient also financial facts in terms of used drugs and other resources available must be considered.

¹The Finnish Register of Visual Impairment 2006.

²Schmidt-Erfurth et al. *Acta Ophthalmol Scand* 2007;85:486-494.

³Rosenfeld et al. *N Engl J Med* 2006;355:1419-1431.

Is it possible to avoid age-related macular degeneration (AMD) ?

Lotta Salminen, Professor, MD

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Over the past 40 years epidemiological studies have illuminated the incidence, prevalence and exposure of AMD within various populations, and confirmed hypothesis on etiology. All studies show the increased risk of the disease with increasing age. People aged 90 years and above have an 8-to 10-fold increased risk of developing AMD compared to people aged 50 years. In addition to the age "inborn" risks are family history of AMD, light skin and female gender. However, studies on distribution of AMD within the population have shown that there are several external and behavioral risk factors of AMD. Social class is reported to have an inverse risk of AMD. Cardiovascular disease, hypertension, carotid atherosclerosis, high intake of saturated fat and cholesterol, smoking, and excessive alcohol consumption have all been shown to associated with increased risk of AMD (Evans 2001).

There are several studies reporting of lifetime light exposure as a risk factor of AMD. The oxidative stress throughout life due to combined exposures to light and oxygen of photoreceptors in the retina and the generation of free radicals may be an essential mechanism for AMD. It is thought that AMD might occur in individuals that have received excess light stimulation, particularly at vulnerable times, or who do not have enough protective antioxidant micronutrients in the serum or retina. Biomarkers of oxidative damage have been demonstrated in postmortem eyes from patients with AMD.

Consequently, the control and treatment of the external and behavioral risk factors of AMD like high blood pressure, excessive weight and obesity, smoking, excessive sunlight exposure may proactively reduce the risk of AMD. The impact of nutrition on manifestation and progression of AMD has become an important,

controversial topic within recent years. The results of the only prospective, controlled, clinical trial providing proven benefit of antioxidant supplementation for AMD (AREDS), and the role of lutein and zeaxanthin and various fatty acids will be discussed during the presentation.

To avoid AMD further research on the interactions between genes and environment is likely to be the most productive way together with balanced, low-fat diet with vitamin supplementation and cessation of smoking.

Low vision aids

Markku Leinonen, MD

Turku University Hospital, Department of Ophthalmology, Finland

Vision can be defective in many ways: visual acuity or the detection resolution of small details can be reduced, the visual field can be narrower than normal or there can be blind areas somewhere in the visual field, the adaptation to various illumination levels can be insufficient or the ability to aim your eyes can be inaccurate. That is why there is so many different kind of defective vision. The functional disability which is caused to the low vision individual varies also depending on the surrounding he is living in and the tasks he must manage in his everyday life.

With the help of personal low vision aids and taking to account the accessibility in all public, and also in private, construction of physical and electronical environment, the coping of a low vision individual in the society can be helped. The visual, auditory and tactile guides of the public environment, routes and cash machines and automatic terminals designed to help people must be constructed to be accessible for also low vision individuals. Web sites have to be developed according to Web Content Accessibility Guidelines because public services have been transferred to electronical form in the internet.

With the help of personal low vision aids the visually impaired can, when mastering the use of the equipment, cope with various situations much better than without the aid. The great number of the low vision aids can become a problem.

The most common optical visual aids are various kinds of magnifying glasses and loupes which can have also own light source. Everyday home life can be much easier with talking kitchen scales, liquid level indicator or electronical color sensor.

If the vision is very poor electronical magnifiers (Closed Circuit TV, CCTV) are needed. The picture from the camera head can be enlarged up to 15 times for the

display. There are several designs of the equipment from pocket size to tabletop machine.

Modern computer in its basic form can be of great value to mildly visually impaired person. Usually the text size of the display can be magnified easily and the color of the text and the background can be adjusted to be suitable for your own vision. You can install special speech synthesizer software which reads aloud the text on the display. More versatile is screenreading software, which can read also the titles of the various windows and the menus of the programs. If you connect a Braille display to the computer you can read with your fingertips the computer text even if you are totally blind.

A small document camera can be connected to laptop computer as an electronical magnifier. It can be aimed to the blackboard of the classroom or to a book besides the laptop.

Modern navigators can help your route with speech assistance when you are walking in unfamiliar district.

Artificial vision with the help of retina implant is only in the beginning of the first clinical trials. If the project succeeds in its goals, a formerly blind person can walk with the help of his new vision and possibly recognize faces or words.

Notes.

Plenary session: Ideas of pathophysiology and future treatment of RD

Vision in Blind Subjects Implanted With Second Sight Medical Products Epiretinal Prosthesis

Mark S. Humayun, Professor of Ophthalmology
Doheny Eye Institute, USC, Los Angeles, USA

M.S. Humayun 1, Jim Weiland 1, M.J. McMahon², A.Caspi², J.D. Dorn², K.H. McClure², R.J. Greenberg².

1 Doheny Eye Institute, University of Southern California, Los Angeles, CA.

2 Second Sight Medical Products, Inc., Sylmar, CA;

Purpose: To demonstrate the level of vision provided by Second Sight Medical Products Epiretinal Prosthesis to patients blind from end-stage retinal degenerations.

Methods: Up to Four Different subjects were asked to do the following tasks: 1) static object differentiation in a high contrast environment, 2) localize electrically elicited visual percepts (phosphenes), and 3) discriminate the orientation of different square-wave gratings.

Results: The subjects performed significantly better than chance in 83% of the tests. Using the video camera, subjects scored as follows on simple visual tasks: locate and count objects (77% to 100%), differentiate three objects (63% to 73%), determine the orientation of a capital L (50% to 77%), and differentiate four directions of a moving object (40% to 90%). The spatial position of phosphenes was consistent with the retinotopic location of the stimulating electrodes. The repeatability of the position judgments and the spatial resolution of the individual electrode positions were similar to the results obtained for sighted subjects who reported the location of analogous visual targets in the absence of visual feedback. When the prosthesis system was used with one subject to view patterned stimuli, orientation discrimination performance was greater than chance (i.e. 25% correct) for gratings finer than two grating cycle per array width, indicating the use of spatial information from individual electrodes.

Results show that for spatial frequencies up to the limit determined by the electrode spacing, performance was significantly above chance.

Conclusions: Blind subjects can use Second Sight Medical Products Epiretinal Prosthesis to differentiate and localize object in their environment. These results,

obtained using a variety of techniques, suggest that the development of retinal prosthesis systems with more electrodes may provide higher spatial resolution vision to blind subjects.

Commercial Relationship: M.J. McMahon, Second Sight, E; A. Caspi, Second Sight, E; J.D. Dorn, Second Sight, E; K.H. McClure, Second Sight, E; M.S. Humayun, Second Sight, F; Second Sight, I; Second Sight, C; R.J.Greenberg, Second Sight, E.

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Stem Cells: Renewed Vision

Maria-Thereza Perez, Associate Professor

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Stem cells and progenitor cells can be programmed to generate multiple cell types. Several reports suggest also that cells with regenerative potential may be isolated not only from the inner cell mass of blastocysts (embryonic stem cells), but also from adult tissues (adult or somatic stem cells). Successful approaches based on the use of such cells would thus offer enormous possibilities to treat a vast range of diseases.

In the area of retinal degenerations, however, the work is not quite yet ready to be tested on human patients. Experimental studies have shown that stem cells and some ocular progenitor cells are capable of differentiating into specific retinal cell types both *in vitro* and following transplantation. However, a number of technical obstacles need still to be resolved before stem cells can be safely used for therapeutic purposes. *In vitro* manipulations are necessary in order to derive and maintain stem cells and involve at present the use of protocols that limit the clinical use of these cells (e.g., contamination with animal pathogens). The potential uncontrolled growth of cells after transplantation needs also to be carefully assessed.

Another approach involves promoting an *in situ* expansion of the small existing pool of endogenous retinal stem or progenitor cells. It would not only eliminate the need to harvest and transplant the cells, but also circumvent the immunological challenges associated with transplantation. This approach requires though a better understanding of the mechanisms controlling normal cell development and thereby of exogenous factors that can be used to boost neurogenesis, since the existing

pools appear unable to contribute spontaneously to any degree of repair. Whether or not application of exogenous factors will also be able to drive the migration of these cells to affected areas and to promote their functional integration with the rest of the tissue remains also unknown.

Nevertheless, despite practical and ethical issues and the fact that many optimistic reports have been refuted, the stem cell field is evolving rapidly, engaging social and technical interests. It has certainly challenged the old dogmas stating that regeneration and repair of the human CNS are not viable.

Efforts need to be intensified in order to improve the prospects of developing effective therapies based on the use of stem and progenitor cells, but avoiding at the same time uncritical enthusiasm and views, which will ultimately delay the advancement of this promising research area.

Clinical Trials: How are they conducted, where and why?

Gerald J. Chader, Ph.D.

Doheny Retina Institute, USC School of Medicine, Los Angeles, CA, USA

Clinical trials are formal research studies that test how well a new approach will be to a medical problem such as inherited retinal degeneration. Some of the areas that can be tested are in 1) prevention options, 2) new diagnostic or screening methods, 3) new treatments (e.g. drugs, devices) or ways to use established treatments in a novel way. Generally, these are preceded by testing at the laboratory bench and in animal models of the relevant disease. The two most important elements in a trial are testing for safety and efficacy, i.e., is the prospective treatment safe in humans and does it actually work. Clinical Trials are conducted according to a strict plan called a Protocol. Most Trials consist of three separate phases although additional phases are sometimes needed to complete the study. Trials are closely regulated by government agencies such as the Food & Drug Administration (FDA) in the United States. Before government approval, however, doctors involved with the Trial must present whatever evidence they have on safety and efficacy to a Review Board or Ethics Committee to justify starting the Trial.

Different groupings such as pharmaceutical companies or government agencies usually fund Clinical Trials and they are managed by a Principal Investigator, usually a medical doctor and staff with experience in conducting such Trials. One or more sites are chosen to conduct the Trial based on facilities and patients available at the site. These sites are usually academic institutions such as medical schools who solicit Volunteers for the Trial from within their patient pool. The

volunteers are usually divided into two groups. The first group receives the actual treatment (e.g., drug, device, etc). The second group does not get the treatment or gets a standard treatment already approved for the disease. In this way, they act as a control (placebo) group for comparison.

In Phase 1 of a Trial, safety is the main issue. A small number of patients are selected to determine if the treatment is safe for use in the human. If a drug or other such agent is involved, often Dose Escalation is studied where increasing doses of the drug/agent are used to assess the safe range for final use in the remaining phases of the Trial. In Phase 2, both safety and efficacy are assessed. A larger number of patients are used here than in Phase 1. Specific tests are conducted to determine if the technique, device or agent is indeed helping the patient. For example, in many trials on the eye, an improvement in Visual Acuity is used as the End Point. In phase 3, an even larger number of patients are used to further assess safety and efficacy. If at the end of all of this, the Trial finds that the treatment is safe, has a beneficial effect and is superior to standard treatment, it can be approved for general use.

The good news for patients with Retinal Degenerations is that Clinical Trials are in progress or soon to start on a large number of treatments that will slow or even cure the degenerative process. For Retinitis Pigmentosa (RP), these include trials on Gene Therapy, Pharmaceutical Therapy (e.g., use of neurotrophic agents), Nutritional Therapy (e.g., antioxidant supplements), Stem Cell Transplantation and use of Retinal Electrical Prostheses. Many of these approaches will also be useful for dry Age-Related Macular Degeneration (AMD). For wet AMD, Clinical Trials have already been positive for Lucentis, an agent that stops new blood vessel proliferation and Trials are in progress or planned for several other similar agents. All these treatments have already passed or yet need to pass through a Clinical Trial before they can be used on general patient populations.

An animal model for AMD

Joe G. Hollyfield, Professor, Ph.D. Director
FFB Research Center, Cole Eye Institute, the Cleveland Clinic Foundation, USA

The signals from the outer retina that bring on AMD are unknown, but are expected to involve epitopes that initiate an immune response. We have developed a mouse model that undergoes AMD-like lesions following immunization with mouse serum albumin adducted with carboxyethylpyrrole (CEP), an oxidation by-product of docosahexaenoic acid present in eye tissue and plasma of AMD patients. Immunized mice develop antibodies to CEP; fix complement component 3 in Bruch's membrane; accumulate debris below the retinal pigment epithelium; and develop focal retinal pigment epithelium lesions

that mimic the blinding end-stage atrophy of dry age-related macular degeneration. These systemically immunized mice are sensitized to the CEP-epitopes generated in the outer retina where docosahexaenoic acid is abundant and the conditions for oxidative damage are permissive. This new animal model for AMD will be important in defining the early events in the disease process, as well as providing a platform for pharmaceutical testing.

Notes.

Saturday 5th July 2008

Plenary session

Visual perception and daily living

Markku Leinonen, MD

Turku University Hospital, Department of Ophthalmology, Finland

Is my vision normal? How a person with visual impairment sees the world? Finding out the deficiencies of your own vision and describing them to other person is difficult. Vision can be defective in many ways: visual acuity or the detection resolution of small details can be reduced, the visual field can be narrower than normal or there can be blind areas somewhere in the visual field, the adaptation to various illumination levels can be insufficient or the ability to aim your eyes can be inaccurate. Conscious vision is mostly brain activity and therefore brain diseases can cause very personal and surprising defects to vision. That is why there are so many kinds of visual impairments.

Normal, conscious vision is basically a huge series of still pictures: the eyes are aimed to a single point for a moment (the fixation), the visual target is memorized and almost at the same time the eyes are directed to a new point which has been selected beforehand. During a normal day a person can have 150 000 – 200 000 these kinds of fixations. With the help of series of numerous fixations a man can utilize the tiny accurate area of his visual field, the fovea which is only 1-2 degrees in diameter. With this complex collaboration of sharp foveal vision and highly accurate series of fixations is composed the illusion which we all share: the illusion of sharp visual field from side to side.

In this talk normal and defective vision is illustrated with the help of computer animations.

Visual field defects and phenomena

Lea Hyvärinen, MD, Senior Lecturer

University of Helsinki and University of Dortmund

Introduction and methods: Visual field defects develop slowly in retinitis pigmentosa, usually as a ring scotoma that later increases to leave a central visual field and peripheral crescents. The defects are measured with Goldmann and automated perimeters, and as confrontation fields using flickering and non-flickering lights, objects and tester's finger movements. The subjectively

experienced light phenomena or the effect of small bright light sources on the functioning of the visual field cannot be measured.

Results: Depending on the measurement technique, the results depict the size and the structure of the visual field in a varying way. The reasons for the variation are:

1) The function of the retina in RP is dependent on the luminance level in a way different from a normal retina. At the low luminance level used in the clinical perimeters (7-10cd/m²) the areas of the scotomas are larger than the scotomas are in room light and much larger than they are in daylight.

2) Exposure to bright light before the examination prolongs the adaptation to the low luminance in the perimeter. Therefore the measurement may show major loss of visual field on a sunny day but this "loss" is not present if the measurement is repeated on a cloudy day or the subject spends several hours in a dimly lit room before the measurement. The variations in the form and size of the visual field are thus difficult to use in assessment of the effects of treatment. Only if strictly standardized adaptation is possible before each measurement, the results are comparable.

3) Since the laboratory measurements do not depict the functional visual field at higher luminance levels, confrontation visual fields should be measured during daily tasks, especially during communication and moving. Sign language communication requires that the person signing knows the extent of the signing space at the distance from the person(s) receiving the information. The size of the signing field depends on the amount of light on the signer and the contrast between the hands and the clothing. The readability of fingerspelling depends also on motion perception, which may decrease at the time when the central visual field is still 30-40 degrees in diameter.

An example of the differences between the results of different measurement techniques:

The visual field measured with a stimulus flickering at 10Hz: the diameter was 180°.

Goldmann perimeter, the two fields drawn to depict the binocular field: diameter was 120° with small scotomatous areas that were covered with the better functioning areas of the other eye.

The communication field was measured in the usual room light. The blouse of the tester was light baize, thus close to the colour of her hands. Therefore the diameter of the communication field at the distance of 120 cm was only 40cm, equal to 20°.

Discussion: In RP, visual fields should be measured using several techniques and luminance levels for a comprehensive assessment of their size and structure.

Science block 3: How to answer your RD patient's questions (English only, no interpretation)

Anti-oxidants, Vitamins and RP

Theo van Veen, Professor
University of Lund, Sweden

In the normal living cell, environmental insults are coped with through a defense or repair mechanism. Whether a cell decides to live or to die is depending on the effectiveness of these mechanisms. Gene mutations influencing the normal cellular processes can affect these mechanisms in a way that the defense is no longer sufficient and the cell decides to die by inducing a series of chemical reactions leading to its death.

Most of inherited forms of photoreceptor degenerations, such as Retinitis Pigmentosa (RP), are the result of a gene mutation that fatally affects the rod photoreceptors, and subsequently, the cone photoreceptors, even though these do not express the mutated gene. A large number of the genes whose mutations are responsible for individual RP genotypes have been identified, and gene therapy clinical trials (LCA) are underway. One constraint on gene therapy, however, is that only an estimated half of all the genes causing the different forms of photoreceptor degeneration have been identified, and it takes long time before all patients have access to gene therapy for their specific mutation.

Neuroprotection (pharmacological interference)

A more general treatment strategy, which does not require identification of the specific genetic factor is the use of neurotrophic factors, since many of the underlying mechanisms for damage to photoreceptors can have common components. It has as goal to halt or delay photoreceptor dysfunction or death, creating a window of opportunity for later on developed cures.

Free radical trappers/scavengers (anti oxidants and vitamins)

Hitherto nine different animal models for RP and post mortem material of AMD patients have shown oxidative DNA damage in their photoreceptors, which seems to be a link the decay process of the cell. Several studies on animal models of RP have shown, however, that combinations of strong anti oxidants can delay photoreceptor cell death.

The reason for oxidative DNA damage could be explained by the results of studies in animal models that the photoreceptor layer becomes hyperoxic in the progression of the disease. Deleterious reactive oxygen species are produced in this hyperoxic environment both metabolically, and by the action of light on photoreceptor compounds. In addition to hyperoxia, oxidative stress can also be triggered by upstream mechanisms that include genetic defects or lack of neurotrophic support. These could directly affect mitochondrial metabolism leading

to increased production of reactive oxygen species and oxidative stress. A gene that is involved in the defense mechanisms of the cell is the transcription factor cAMP-response-element-binding (CREB). We have shown that CREB protein is down-regulated which leads to down-regulation of CREB target genes including 8-oxoguanine DNA glycosylase (OGG1), an enzyme critical for the repair of oxidatively damaged DNA, and calpastatin, the endogenous inhibitor of calpain-type calcium-activated proteases. These events all cause an increase in oxidatively damaged DNA and hence over activation of enzymes engaged in DNA repair such as poly-ADP-ribose-polymerase (PARP). Over activation of PARP leads to additional oxidative damage and translocation of Apoptosis Inducing Factor (AIF) to the nucleus where it initiates massive DNA damage and cell death. Interfering with these processes using high concentrations of free radical scavengers could thus lower the oxidative stress level and therewith delay the resulting cell decay.

What are patient registries and why are they needed?

Eberhart Zrenner¹, Robert Wilke¹, Eric Tröger¹, Torsten Strasser¹

¹Centre for Ophthalmology, Institute for Ophthalmic Research, University of Tübingen, Germany

In the Western hemisphere practically every patient suffering from hereditary retinal diseases is seen by an ophthalmologist. Although numerous data are available, patients are rarely listed in central databases that would allow utilizing these data for scientific projects. The establishment of patient registries and the entry of patient data on the other hand can be of great benefit not only for the research in hereditary retinal degeneration, but also for the patient for the following reasons:

- Epidemiological research: The prevalence and incidents of the various subgroups of hereditary retinal diseases can be assessed; factors that aggravate the disease may be discovered.
- Clinical research: The differentiation of the various subgroups and discoveries of diagnostic markers as well as the typical course of disease can be studied.
- Genotype-phenotype correlation: In cooperation with geneticists, the genetic basis of disorders in individual patients can be described and a relation to a phenotype, i.e. the clinical characteristics of the particular subgroup, can be uncovered.

- Suited patient groups can be recruited for clinical trials: This is very important at a time where pharmacological therapies become available, gene replacement methods are applied and retinal implant studies are being performed.
- In conjunction with tissue banks proteomics can be applied: The investigation of altered proteins may lead in the future to new therapies.

So far data bases are scattered and several centers have been established worldwide. In Europe, within the “Integrated project EVI Genoret”, applied by the European Vision Institute (<http://www.europeanvisioninstitute.org>) a strong effort has been made to harmonize the entries into a central database in clinical ophthalmology and genetics by means of a three level data base:

1. Level 1 covers the essential data that is stored on a central computer so that (strictly anonymised) patient groups with certain diagnosis or gene mutations can be found.
2. Level 2 is only accessible after proper arrangements between investigator and physicians. Having admission to individual patients the full (still anonymised) data set can be utilized. The contact to the patient is exclusively made by the treating physician.
3. Level 3 of the EVI Genoret data base contains special investigations, performed within research projects. It is recommended that patients registers through the various European centres attach to the EVI Genoret database (for more information see <http://www.evi-genoret.org>)

Rehabilitation

Sirkka-Liisa Rudanko, MD, Chief Ophthalmologist
Finnish Federation of the Visually Impaired

Disability due to progressive inherited retinal dystrophies can be significantly alleviated by efficient rehabilitation. Common problems associated with inherited retinal dystrophies are night blindness, slow adaptation to changes in illumination, light sensitivity, “tunnel vision” or large central scotomas in visual fields. Considerable visual fluctuation, general visual haze or various flying and sparking lights in visual fields may appear frequently or even constantly. Difficulties at activities of daily living, mobility, reading and communication can be diminished by various methods developed for the visually impaired of the modern society.

A multitude of rehabilitation measures are available, starting from visual rehabilitation and training of daily living activities, mobility functions and the use of

modern information technology with adaptive aids. Environmental adjustments, assistant services (e.g. guides and interpreters), social support and support for education and employment are also arranged.

New medical imaging technology has shown significant cerebral activation during visual and multisensory training. Multiple cerebrovisual adaptive changes have also been discovered along systematic training, and consequent functional improvement has been proved both in children and adults. Crucial improvements in visual and other functions have been achieved by using visual aids, new viewing techniques, individually suitable lighting and ergonomics, and by using various other devices and adaptive aids developed for low vision and blind people. In addition, a multitude of multisensory, compensatory and supporting techniques have presented valuable roles in rehabilitation processes.

The efficiency and value of systematic rehabilitation for visual impairment have been thoroughly verified by recent studies. Long-term rehabilitation, including assessment of functional profile and follow-up, is still the key procedure for taking care of people with severe retinal dystrophies.

Notes.

Patient block 4: Rehabilitation (English-Finnish interpretation)

Career Choice and Vocational Rehabilitation in Finland

Taru Tammi, Head of the Employment Services Department
Finnish Federation of the Visually Impaired

Support at the stage of career choice aims to help visually impaired person to build his/her own individual path to working life. The visual disability and its prognosis have to be taken into account when choosing career and education, but the most important thing is to support the individual to understand his/her own identity and strengths. The vocational counselling begins at clarifying opportunities and seeking solutions. The goal of the process is to find the most suitable occupation and tasks for each.

The vocational counselling consists of support at the stage of choosing profession and education and of support to find a place in working life. Counselling process begins with guidance discussion with employment advisor. Visually impaired young persons often have limited knowledge of career possibilities because lack of work experience and lack of education. The process of vocational rehabilitation begins with discussion about expectations, motivation, skills and interests. If the person already has previous work experience and/or education these have to be taken into account in planning the next steps. The guidance discussion also includes assessment of visual disability and its influence on working capacity.

The means of vocational rehabilitation supporting choice of career and finding employment include: work and training try-outs, training designed to maintain and enhance work capacity, basic vocational training, skills updating or retraining, assistive devices for work and study and arrangements in working environment. When necessary the vocational rehabilitation is supported by individual social rehabilitation, low vision training and training in orientation and mobility.

At the stage of the career choice for person with retinal dystrophies is important to pay attention to the nature of work and tasks as well as to the working environment. The possibility to have own workroom, where lighting and other working conditions are optimal enables working at many occupations and tasks.

Patient block 6: Usher syndrome (USH) (English-Finnish interpretation)

Gene therapy of USH 3

John Flannery, Professor

The Helen Wills Neuroscience Institute, University of California, Berkeley, USA

Major progress in understanding the molecular causes of Usher syndrome has been made in the last few years. Usher syndrome was initially categorized by its clinical appearance as two distinct types of autosomal recessive deaf-blindness: Usher I patients had profound congenital sensorineural deafness, vestibular dysfunction and retinitis pigmentosa (RP). Usher type II patients had moderate congenital hearing impairment, normal vestibular function, and RP. A third USH subtype was later identified as clinical ophthalmologists noted progressive hearing loss in some patients previously diagnosed with RP. At the same time, molecular geneticists identified a gene defect in (Clarin-1, CLRN1), a causative gene distinct from the other Usher subtypes. Usher type 3 is now universally accepted as a separate disease subtype characterized by progressive (non congenital) hearing loss, variable vestibular abnormality and RP. Usher 3a is relatively rare in most parts of the world; however, it represents about 40% of Usher patients in Finland and in Ashkenazi Jews in Europe and North America.

The structure of the clarin-1 gene (CLRN1) is now known and its coding sequence comprises 699 base pairs. The protein encoded by the gene is a tetraspanin, with a hypothesized function in sorting and trafficking proteins to the rod photoreceptor outer segment through the connecting cilium. The small size of the clarin coding sequence makes it a highly attractive candidate for gene therapy because it may be delivered using the recombinant Adeno associated virus (AAV) technology. The safety and efficacy of using AAV vectors for gene therapy for recessive retinal degeneration has recently achieved proof of concept in the three clinical trials for Leber's Congenital Amaurosis, an autosomal recessive retinal degeneration. Usher 3 is also an autosomal recessive disease and one potential treatment will be to deliver a normal copy of the mutated clarin -1 gene to the photoreceptor cells using AAV vectors. Usher 3a is the slowest progressing of the three types of Usher syndrome, which increases the time window into which a gene replacement therapy may be applied.

John Flannery, Scott Geller

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Science block 4: RD Research in Europe (English only, no interpretation)

Retinal degeneration research in Europe

Eberhart Zrenner, Professor

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Research in retinal degeneration has been developed in many countries in Europe very well. The annual meeting on retinal degeneration, held in Potsdam every April in the meantime has usually 200 attendants from all European countries and numerous presentations on the genetics, the molecular biology, the proteomics, the clinical studies on the various subgroups are given at this meeting as well as at other European meetings such as EVER (www.ever.be), EURETINA (www.euretina.org), SOE and many other national scientific meetings. Through these meetings a lively network of RD-researchers has been established in Europe and numerous examples of recent accomplishment in gene mutation discovery, animal model research, clinical studies and therapy approaches will be given.

There is a special activity, supported by the European Union by 10 million Euro entitled EVI GENORET (Functional Genomics of the Retina in Health and Disease): 25 academic and industrial partners from five interacting components (phenotyping, genotyping, development, therapy and functional gene analysis) have established a working platform, share tools and knowledge within and outside the academic community, including patient organisations and industrial partners. The particular aims are: (1) Obtaining the information provided by the clinical conditions and animal models; (2) Analysis of the information: Functional genomic tools; (3) Validation of the information and (4) Design of genomic based therapy.

This year's highlight is a new activity supported by the European Community. The project 'EuroVisionNet' (Visual Impairment and Degeneration: A Roadmap for Vision Research within Europe; www.eurovisionnet.eu), coordinated by the European Vision Institute (www.europeanvisioninstitute.org), will open a new chapter of integration and cooperation of Vision Research throughout Europe. The major aim of this project is to consolidate vision research activities and policies of the European Vision Research community, in order to overcome still existing fragmentation, in particular by providing a web-based portal to considerably improve communication and information exchange in the field. A major breakthrough in this approach is the active participation of patient organisations represented by Retina International (www.retina-international.org).

Pharmacological Therapy of RD. An Overview.

Arto Urtti, Professor

Centre for Drug Research, University of Helsinki, Finland

Pharmacological therapy of the retinal degenerations has advanced during this decade significantly, and some of the future prospects are also promising. Drug treatment of the age-related retinal degenerations is based on intravitreal injections of aptamer or antibody drugs that are inhibiting the neovascularization. Particularly the antibodies have shown beneficial effects, and are gaining increased use clinically. Photodynamic therapy is also used but its efficacy does not match that of the antibodies. The future prospects include: 1) prolonged action controlled release devices for the trans-scleral drug delivery into the retina, 2) gene therapy, and 3) cell therapy with microencapsulated cells that produce the therapeutic protein continuously. Current medications and future prospects will be discussed in the presentation.

Results from the Subretinal Implant Trial: Visual Sensations Mediated By Microelectrode Arrays Implanted Into Blind Retinitis Pigmentosa Patients

Eberhart Zrenner, Professor

Centre for Ophthalmology, Institute for Ophthalmic Research, University of Tübingen, Germany

Subretinal implants, consisting of a chip (3x3x0.1mm, 1500 microphotodiodes, amplifiers and electrodes of 50x50µm, spaced 70µm) and a 4x4 array of identical electrodes, spaced 280µm, for direct stimulation (DS) were chronically implanted next to the foveal rim in patients. The implant was in proper place in all patients; none of the patients suffered from serious events such as infections, retinal detachment, major bleeding or pain. Chip and DS array are positioned on a small subretinal polyimide foil powered via a subretinal transchoroidal, retroauricular transdermal line. Visual perception of brightness elicited by applying biphasic voltage impulses from 1 to 2,5V (t = 3 ms) was assessed using a scale from 5 (very strong) to 0 (none); additionally double impulses with differences up to 0.8V between two stimuli (10 s interval) were applied.

Results: Electrical stimulation of rows, columns and blocks of 4 electrodes allowed some patients to clearly distinguish horizontal from vertical lines and positions, respectively. Under optimal conditions, dot alignment and direction of dot movement was properly recognized, if three neighbouring electrodes were switched on simultaneously or sequentially at 1 s intervals. Brightness perception

of spots varied from scale 0 to 5 in a linear manner if voltages between 1.5 and 2.5 were applied (randomly 6 times) to a square of 4 electrodes. This corresponds to a charge increase of approximately 0.23 mC/cm² for each of the 5 steps. A difference in brightness between two consecutive pulses was discerned, if a difference in charge of at least 16 µC/cm² was applied. If equal charges were applied to both conditions, the second flash always was perceived slightly dimmer irrespective of the stimulation level. Subjective brightness amplification phenomena were observed at medium stimulation levels and at certain frequencies. The subjective size of spot perception upon stimulation of a square of 4 electrodes increased from 1 to 5 mm at arms length, if the voltage was increased from 1,5 to 2,5 V. In SLO microperimetry of the chip, single light spots down to 100 to 400 µm in diameter were detected, allowing the patient to localize a white plate on a black table cloth correctly.

Conclusions: Subretinal electrical multielectrode stimulation can provide a useful range of localized brightness perceptions in blind patients within a limited range of temporal, spatial and electrical parameters.

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Patient block 7: Family

Environmental information through Touch – haptices and haptemes

Dr Riitta Lahtinen, Finnish Deafblind Association &
Russ Palmer, Music Therapist

Haptices is based on PhD research of how dual-sensory impaired people can share environmental information interactively through touch. The research is systematic, longitudinal and development description of how to adapt visual and auditive information to the body. This new approach can be recognised as social-haptic communication which is a form of “touch language”.

Research describes haptices in different situations enhancing sensory information and functioning also as an independent language. Haptices includes confirmation system, social quick messages, body drawing, contact to the people and the environment, guiding and sharing art experiences through movements. Haptices give the possibility to share emotional experiences and atmospheres, different hobby and game experiences.

Haptices are made from haptemes that determines which regulations are analysed. Haptices include sharing a personal body space, meaning of touch-contact, context and using different communication channels. Communication distances are classified as exact distance, estimated distance and touch distance. This research classifies how the body can be identified into different areas such as body orientation, varied body postures, body position levels, social actions and which side of the body is used. Haptemes of movements are recognised as the direction of movements, change of directions on the body, directions between people, pressure, speed, frequency, size, length, duration, pause, change of rhythm, shape, macro and micro movements.

Social-haptic communication system is now starting to be appreciated in Scandinavian and parts of Europe by different client groups i.e. Deafblind people, visually impaired adults and children, teachers and interpreters.

Riitta & Russ: "As a couple for the past 17 years we have analysed how we can obtain the visual clues from the environment such in order to appreciate interactive social and art experiences. This included different levels of environmental information such as a lay out of the different rooms, objects or outlining a specific route. This approach save a lot of time wasting, saves energy, transmitting confirmation and non-verbal messages and enabling private messages between two people. For example when we enter to the auditorium by using guiding grip:

Riitta: "Auditorium." Russ nods and looks around.

Riitta draws the ground plan of the auditorium onto the back of Russ, then showing and saying the landmarks of auditorium:

"Door – lecturer – free seats."

Russ: "Let's stay here beside the door."

Riitta shows the route onto the back.

Reference: Lahtinen, R. 2008. Haptices and haptemes. A case study of developmental process in touch-based communication of acquired deafblind people. Doctorial theses, Helsinki University, Special Education. Tampere: Cityoffset.

Notes.

Patient block 8: RD research in Europe

What are patient registries and why are they needed?

Sirkka-Liisa Rudanko, MD, Chief Ophthalmologist
Finnish Federation of the Visually Impaired

PATIENT REGISTRIES

A patient register is a medical datum for a certain particular purpose, e.g. for patient services or scientific purposes. In addition to patient registers in every health care unit, data are also collected by national health care registries established for special important reasons. National registries and their international cross-border co-operation is of crucial importance for analyzing, following and solving difficult problems associated with severe diseases.

Our knowledge on genetic eye diseases has increased enormously during the last two decades. Gene therapy research is going on in laboratories around the world and promising results have already been obtained. Lots of clinical trials need still to be performed for clarifying benefits, safety and long-term efficiency achievable by gene transfers.

Specific gene mutations need to be localized and identified for gene transfers. Clinical trials have been started with relatively easily recognizable defects, e.g. mutations detected in retinitis pigmentosa, Usher syndrome and Leber congenital amaurosis. "Phenotyping-genotyping" studies are now very important for detecting correlations between clinical signs/symptoms and gene mutations, thus facilitating the recognition of mutations.

In Finland, special attention has been paid to eye diseases within the Finnish Disease Heritage, including also neuronal ceroid lipofuscinosis, x-linked juvenile retinoschisis, choroideremia and gyrate atrophy. Wide national variations occur in disease genes, why particular Finnish gene mutations need also to be known prior to gene therapeutic interventions in Finland. Fortunately, the relatively homogenic population structure in Finland is beneficial in this matter. There are relatively good opportunities for accurate mapping of genetical eye diseases in Finnish people and consequent possibilities for introducing new methods of testing and treatment throughout the country.

Along with the continuous progress in molecular genetics, it is important to enhance national epidemiological, ophthalmological and genetic studies on chorioretinal dystrophies, to organize systematic data gathering and encourage

cross-border co-operation in vision research. There is an obvious need of national patient registries and international scientific co-operation for successful research and development of treatments for retinal dystrophies. We may already be optimistic and believe, that the visual prognosis of people in RD families will essentially improve within the near future by means of new interventions.

Present and Future Treatments for Retinal Degenerative Diseases

Gerald J. Chader, Ph.D.

Doheny Retina Institute, USC Medical School, Los Angeles, CA, USA

More and more is being understood about the basic genetic factors involved in RP and AMD as well as the biological mechanisms that lead to photoreceptor degeneration. For example, it is estimated that about one-half of the genetic mutations leading to RP are known as well as most of the gene mutations involved in AMD. Armed with this genetic information and knowledge of basic mechanisms of photoreceptor cell death, strategies have been designed to slow vision loss or even restore vision. In some cases, the same treatment can be used for RP and dry AMD.

Clinical Trials are now in place or being planned for both AMD and RP – including rare RDs such as Stargardt and Usher Syndrome. General strategies involve 1) Gene Therapy 2) Pharmaceutical Therapy 3) Photoreceptor/Stem Cell Transplantation 4) Nutrition and 5) Retinal Prosthetic Devices. Gene Therapy has shown great success (efficacy and safety) in RD animal models. Clinical Trials are already taking place for a specific form of Leber Congenital Amaurosis. Several trials for other forms of RP including Usher and Stargardt diseases are being planned. A Clinical Trial is in progress (Neurotech Co.) using a Pharmaceutical Therapeutic approach. In this case, a neurotrophic factor (CNTF) is supplied to the retina using a tiny capsule implanted within the eye to slow photoreceptor degeneration. Phase 1 of this Trial has been successfully completed and Phase 2 Trials for RP and for dry AMD are in progress. A Trial for Photoreceptor Transplantation is in place but few good results have been obtained. On the other hand, stem cell transplantation offers great hope for future treatment of both RP and AMD. Various Nutrition strategies are being developed based on the fact that severe oxidative damage seems to occur in the RD retina. Antioxidants have been shown to markedly slow photoreceptor cell death in RP animal models and a Clinical Trial is in place in Spain using a special group of antioxidants (“RetinaComplex”). In cases where all photoreceptors are dead, the Retinal Electrical Prosthesis could restore functional vision. Results on different prosthesis designs from several groups around the world are encouraging. There are early

human Clinical Trials on the prosthetic devices that ultimately could be useful to AMD as well as RP patients.

For wet AMD, pharmaceutical strategies are being employed to halt and possibly reverse damage due to choroidal neovascularization. Lucentis injections, for example, are effective in slowing the wet AMD disease process. For dry AMD, different methods are being employed to slow photoreceptor cell death. Already in use is the antioxidant supplement mixture studied in the Age-Related Eye Disease Study (AREDS) Clinical Trial. A new trial, AREDS2, will test the efficacy of the carotenoids lutein and zeaxanthin in slowing the progression of AMD.

In summary, much is now known about the inherited retinal degenerations. Specifically, enough is known to demonstrate scientific “Proof of Principle” in RD animal models that such interventions can be both efficacious and safe. Based on this, human Clinical Trials for these diseases are now taking place with many more to come in the next few years.

Closing session: Ongoing treatment trials

Update on phase I/II clinical trial of RPE65 gene therapy in adults with Leber congenital amaurosis

Robin Ali , Professor

UCL Institute of Ophthalmology and Moorfields Eye Hospital Biomedical Research Centre for Ophthalmology, London, UK

Early-onset severe retinal dystrophy caused by defects in the gene encoding the retinal isomerase RPE65 is associated with poor vision at birth and complete loss of vision in early adulthood. In February 2007 we started a phase I/II clinical trial of gene therapy in 3 young adult subjects. We administered subretinally a rAAV-2/2 vector expressing RPE65 cDNA under the control of a human RPE65 promoter. Examination of systemic vector dissemination, immune responses, electrophysiology, retinal imaging and detailed psychophysical assessments of visual function suggest that subretinal administration of rAAV vector is safe in humans and can lead to improved visual function. These findings support further clinical studies in children with RPE65 deficiency and the development of gene therapy for other inherited retinal disorders.

Phase II/III Studies of ECT-CNTF in patients with dry AMD or Retinitis Pigmentosa

Weng Tao, M.D., Ph.D.

Neurotech, USA

Objective

To investigate whether CNTF, delivered via an encapsulated cell technology implant, improves visual function in subjects with dry AMD or retinitis pigmentosa (RP).

Purpose

To evaluate the safety and efficacy of NT-501 in a multi-center, double-masked, controlled, dose-ranging and randomized phase II trial for dry AMD (CNTF2) and phase II/III trials for late RP (CNTF3) or early RP (CNTF4).

Method

The CNTF2 study consists of 48 participants that will be randomized to the higher or lower CNTF output implant, or to sham surgery in a 2:1:1 ratio. The CNTF3 and CNTF4 studies each consist of 60 participants that will be randomized to the higher CNTF output implant and the lower CNTF output implant in a 2:1 ratio. The

contralateral eye will receive sham surgery. These studies will allow safety assessment and the evaluation of primary and secondary efficacy outcomes that may be used in future studies, or in a BLA submission in the case of RP.

Results

To date, the results obtained from implanted subjects suggest that the NT-501 implant and the implant procedure are well tolerated and safe.

Conclusion

Full results for all three studies at the point of the primary efficacy outcome measurement will be available in late 2008. If NT-501 proves to be safe and effective, it would be among the first treatments for dry AMD and RP.

Notes.

POSTERS

PDZK7 DOES NOT PARTICIPATE IN THE USHER PROTEIN NETWORK

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INTRODUCTION

Usher syndrome (USH) is an autosomal recessive genetic disease defined by the association of sensorineural hearing loss and visual impairment due to retinitis pigmentosa (RP), with or without vestibular affectation. USH is clinically and genetically heterogeneous. Nine genes have been identified which codify for proteins that are integrated in a protein network known as Usher interactome. The central core of the interactome is formed by the PDZ domain containing homologues harmonin and whirlin with the rest of USH proteins assembling to this core. Additional interacting proteins assembling to the USH protein network are candidates to be responsible for Usher syndrome, non-syndromic hearing loss or retinal dystrophies.

HYPOTHESIS

The protein product predicted for PDZK7 (NM_024895) shows high homology to both harmonin and whirlin and could have a similar function. Thus, it is a candidate member for the Usher interactome.

OBJETIVE

The aim of the present work was to determine the existence of interactions between PDZK7 and the proteins of the Usher interactome: usherin, NBC3, VLGR1, cadherin 23, protocadherin 15 and SANS.

METHODS

Interactions were tested by yeast two hybrid (Y2H) assays and by co-expression and co-localization in cos-1 cells studies.

RESULTS

No interaction was found between PDZK7 and USH proteins.

CONCLUSIONS

The absence of interactions between PDZK7 and USH proteins indicates that it is not part of the USH interactome.

PROINSULIN ATTENUATES VISION LOSS AND DELAYS PHOTORECEPTOR APOPTOSIS IN A MOUSE MODEL OF RETINITIS PIGMENTOSA

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Retinitis pigmentosa is a heterogeneous group of inherited conditions that lead to blindness and for which there is no effective therapy. Photoreceptor apoptosis is a common feature in animal models of the disease. Here, we investigate the therapeutic potential of proinsulin in the rd10 model, based on its antiapoptotic effect exerted during retinal development. Transgenic mice expressing human proinsulin (hPi) in the skeletal muscle were generated. Two independent lines of hPi/rd10 mice (L1 and L2) were established that constitutively expressed hPi in muscle. In these mice, hPi production was not regulated by glucose levels. hPi levels in serum, muscle and retina were determined by a commercial ELISA kit, visual function was evaluated by electroretinogram recording and programmed cell death was assessed by TUNEL. Immunohistochemistry was used to evaluate oxidative damage and retinal structure preservation. Transgenic expression of hPi in the retinal degeneration mouse model

rd10 extended visual function in a manner that was related to the level of transgene expression. Furthermore, attenuation of visual deterioration correlated with a delay in photoreceptor apoptosis and with preservation of retinal cytoarchitecture, particularly that of the cones. Our findings provide with a new basis for a potential therapy for retinitis pigmentosa, as well as a new tool to identify the mechanisms involved in the progress of retinal neurodegeneration.

EVALUATION OF A GENOTYPING MICROARRAY FOR USHER SYNDROME

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INTRODUCTION

Patients with Usher syndrome (USH) suffer from retinitis pigmentosa (RP), sensorineural hearing loss and some times, vestibular dysfunction. This disease is clinically and genetically heterogeneous. Three clinical subtypes are distinguished: Usher syndrome type I (USH1) combines severe to profound congenital deafness, prepubertal onset of RP and vestibular dysfunction. Patients with Usher syndrome type II (USH2) show moderate to severe hearing loss and RP. Usher syndrome type III (USH3) patients present progressive hearing loss, RP and variable vestibular areflexia. Nine genes are known to be involved in this disease: MYO7A, CDH23, PCDH15, USH1C and USH1G for USH1, USH2A, VLGR1 and DFNB31 in USH2 and USH3A for USH3.

MATERIAL AND METHODS

Genomic DNA from 192 unrelated patients with Usher syndrome was obtained by standard procedures. These patients were classified in 55 USH1, 98 USH2 and 10 USH3. Clinical data was not available for 29 USH patients.

A genotyping microarray for Usher syndrome (Asper Biotech) was designed as a tool for the genetic analysis of USH patients. The USH microarray is based on the APEX (Arrayed Primer Extension) technology and it allows the detection of 250 previously detected mutations in USH genes.

All identified pathologic variants were confirmed by direct sequencing.

OBJECTIVE

The objective of this work is to evaluate the efficacy of the USH genotyping microarray for the detection of known mutations in a cohort of patients of Mediterranean origin suffering from Usher syndrome.

RESULTS

Mutations were identified in 69 out of 192 USH patients (35.9%) and we found the 25.8% of expected mutant alleles.

For USH1, mutations were identified in 17 cases (30.9%) whereas mutated alleles account for 18.2%. For USH2, we found 39 pathologic variants in 98 patients (39.8%) and the 16.3% of mutated alleles were identified. 20% of the USH3 patients (2/10) were found to be carriers of mutations and a 16.3% of pathologic alleles were identified. In USH patients without a clinical classification 11 mutations were found (37.9%) and the percentage of mutated alleles was 24 %.

CONCLUSIONS

The USH microarray is a rapid and versatile method to identify the genetic cause of the USH. However, this technique only allows the detections of known mutations. So, it is important to increase its efficiency by performing constant updates with all identified novel pathologic variants.



A NOVEL CACNA1F GENE SPLICING MUTATION IN A FINNISH NIGHT BLINDNESS PATIENT

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PURPOSE: The CACNA1F gene codes for the alpha1-subunit of Cav1.4 calcium channel. The channel is expressed mainly in retina, and mutations in the encoding gene can cause several retinal diseases, including X-linked incomplete congenital stationary night blindness (CSNB2), retinal and optic disc atrophy, Åland Island eye disease (AIED), and progressive cone-rod dystrophy (CORDX3). The purpose of this study was to determine the mutation underlying the phenotype of a Finnish patient with congenital nystagmus, reduced visual acuity, progressive myopia, astigmatism, photophobia, defective dark adaptation, and pale optic discs and ocular fundi.

METHODS: All 48 exons and flanking intronic regions of the CACNA1F gene were analyzed by direct sequencing. Presence of the identified mutation among control samples was studied with HpyCH4III restriction endonuclease analysis. The exon 14-18 region of CACNA1F mRNA from cultured lymphoblasts was analyzed by RT-PCR and cDNA sequencing.

RESULTS: A novel CACNA1F gene mutation, IVS16+2T>C, in the splice donor site of intron 16 was identified in the patient and his carrier mother, but it was absent from the 100 control chromosomes studied. RT-PCR analysis of lymphoblast RNA from the patient revealed an aberrant splicing product due to mutation predicting in-frame deletion/insertion into the encoded channel protein. Control sample showed a wt transcript that contained all the exons 14-18, as well as novel alternative splice variants predicting in-frame deletion/insertion or premature termination codons.

CONCLUSIONS: Most of the mutations in the CACNA1F gene lead to CSNB2 phenotype. Some patients may, however, show divergent but overlapping phenotypes. The phenotype of our patient was consistent with CSNB2, although it had features also from progressive cone-rod dystrophy, such as the progressive myopia and missing cone threshold in dark adaptation. The observed alternative CACNA1F splice variants, which predict in-frame deletion/insertion or premature termination codons, suggest that alternative splicing is an important means to produce functional diversity to Cav1.4 channel, or down-regulate its expression level.

THE O.N.C.E PROGRAM OF GENETIC COUNSELLING ON INHERITED OCULAR DISORDERS: 7 YEARS OF EXPERIENCE

C.Ayuso – Associate Director of the Genetic Service JDF (Jimenez Diaz Foundation)

I.Lorda – Assistant Doctor Genetic JDF

E.Martin – Ophthalmologist Adviser O.N.C.E

E.Vallespin – Biologist Genetic JDF

C.Villaverde – Laboratory Technician Genetic JDF

PURPOSE: a pilot program to provide genetic counselling on inherited ophthalmologic disorders

METHODS: patients asking for this service, from April 2001 to March 2008. Questionnaire including clinical and pedigree information or personal interview

RESULTS: 627 patients ranging from 1 to 86 years were attended. 330 recessive, 168 dominant, 79 sporadic, 32 x-linked, 10 multifactorial, 6 environment and 2 mitochondrial familiar presentation of the disorder were observed. Main diagnoses were retinal dystrophies (70%) and congenital ocular malformations (10%).

CONCLUSIONS: patients were satisfied with this program since appropriate genetic classification and counselling was achieved in most of the cases.

MUTATION SPECTRUM OF USHER SYNDROME IN FINLAND

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PURPOSE: Finnish epidemiological studies of Usher syndrome (USH) suggested that the distribution of clinical USH types is 40% USH3, 34% USH1, and 12 % USH2 (14% unknown). We previously detected a USH3A (CLRN1) founder mutation in most Finnish patients who were clinically diagnosed with USH3. Due to the considerable overlap of clinical symptoms between the USH types, however, some patients clinically characterized as USH3 did not show CLRN1 mutations. Unlike USH3A, USH1 is heterogenic in Finland. Recently we detected three novel USH1B mutations in two unrelated Finnish USH1 patients. The purpose of this study was to further investigate the USH mutation spectrum in Finnish patients without previously known USH mutations.

Methods: For this study, we analyzed samples from 14 USH patients without previously known mutations using the Asper Genetics USH mutation chip.

RESULTS: From these 14 Finnish USH patients we detected seven different sequence variations in heterozygous form: two in MYO7A, and five in USH2A genes. These variations were not previously known to exist in the Finnish population.

CONCLUSIONS: Mutation chips are useful in detecting mutations in a heterogeneous disease such as USH. Based on our study, there is considerable heterogeneity of USH1 and USH2 in Finland, in contrast to USH3 which is merely caused by a founder mutation. All seven mutations detected in 14 USH patients were in heterozygous form. Although the pathogenicity of these sequence variations is still undetermined, there remains a possibility of digenic or even trigenic inheritance including CLRN1, MYO7A, and USH2A genes.