European School of Genetic Medicine

5th Course in

Eye Genetics

Bertinoro (Italy) September 24-26, 2017

University Residential Centre
Via Frangipane, 6 – Bertinoro
www.ceub.it

Course Directors:
R. Allikmets (Columbia University, New York) A. Ciardella (U.O. Oftalmologia, Policlinico Sant' Orsola, Bologna) B. P. Leroy (Ghent University, Ghent) M. Seri (U.O Genetica Medica, Bologna).

5th Course in

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COURSE PROGRAM

September 24

8:30 - 8:45 **Welcome**
Giovanni Romeo and Rando Allikmets

8:45 - 9:30 **2 parallel talks: (40 min + 5 min discussion)**

Garrison Room

1. Overview of clinical ophthalmology for basic scientists
Antonio Ciardella

Jacopo da Bertinoro Room

2. Overview of basic medical genetics for ophthalmologists
Bart Leroy

9:35 - 11:05 **2 talks (40 min + 5 min discussion)**

3. Stargardt disease, the complex simple retinal disorder
Rando Allikmets

4. Genetics of corneal diseases
Graeme Black

11:05 - 11:30 **Break**

11:30 - 13:00 **2 talks (40 min + 5 min discussion)**

1. Molecular basis of non-syndromic and syndromic retinal and vitreoretinal diseases
Wolfgang Berger

2. Introduction to next-generation sequencing for eye diseases
Susanne Roosing

13:00 - 14:00 **Lunch**

14:00 - 16:00 **3 parallel workshops**

Garrison room
WS1 Preparation: Student discussion group on interesting cases (clinical, molecular, families, etc.) they have encountered (**Graeme Black & Bart Leroy**)

Jacopo da Bertinoro room
WS4 Genetic counseling (**Georgina Hall & Marco Seri**)

Computer room
WS5 Genomics: technological developments and interpretation of results; the impact of next generation sequencing on retinal disease gene identification (**Frans Cremers & Susanne Roosing**)

16:00 - 16:30 **Break**

16:30 - 18:30 **3 parallel workshops**

Garrison Room
WS1 Preparation: Student discussion group on interesting cases (clinical, molecular, families, etc.) they have encountered (**Graeme Black & Bart Leroy**)

Jacopo da Bertinoro room
WS2 Clinical approach to hereditary retinal diseases (**Antonio Ciardella, Claudio Graziano, Andrea Sodi**)

Computer room
WS3 Disease-causing mutations: finding and interpretation (**Wolfgang Berger & Rando Allikmets**)

September 25

9:00 - 11:15 **3 talks (40 min + 5 min discussion)**

1. Genetics of RP/LCA/CSNB
   Bart Leroy

2. Stem cells in eye diseases
   Jane Sowden

3. Genetics of age-related macular degeneration
   Rando Allikmets

11:15 - 11:45 **Break**

11:45 - 13:15 **2 talks (40 min + 5 min discussion)**

4. Overview of developmental eye anomalies
   Graeme Black

5. Retinal ciliopathies: diverse phenotypes with overlapping genetic structure
   Nicholas Katsanis

13:15 - 14:15 **Lunch**

14:15 - 16:15 **3 parallel workshops**

Jacopo da Bertinoro Room
WS2 Clinical approach to hereditary retinal diseases (**Antonio Ciardella, Claudio Graziano, Andrea Sodi)**

Garrison Room
WS4 Genetic counseling (**Georgina Hall &Marco Seri**)

Computer room
WS3 Disease-causing mutations: finding and interpretation (**Wolfgang Berger & Rando Allikmets)**

16:15 - 16:45 **Break**

16:45 - 18:45 **2 parallel workshops**

Jacopo da Bertinoro Room

WS1 Final preparation for student presentations and selection of 10-12 cases for presentation (Graeme **Black & Bart Leroy**)

Computer room

WS5 Genomics: technological developments and interpretation of results; the impact of next generation sequencing on retinal disease gene identification (**Frans Cremers & Susanne Roosing**)

September 26

9:00 - 11:15 **3 talks (40 min + 5 min discussion)**

1. Architecture of genetic disease: causes, modifiers and the concept of genetic load
   Nicholas Katsanis

2. Genetics of glaucoma
   Jane Sowden

3. Gene therapy for recessive and dominant eye disorders
   Alberto Auricchio

11:15 - 11:45 **Break**

11:45 - 13:15 **2 talks (40 min + 5 min discussion)**

4. The role for non-coding RNAs in eye development, function and diseases
   Sandro Banfi

5. Modifier genes in retinal diseases
   Frans Cremers

13:15 - 14:15 **Lunch**

14:15 - 15:45 **Student presentations**

15:45 - 16:15 **Break**

16:15 - 17:45 **3 shorter talks (25 min +5 min discussion)**

6. Genetics of mitochondrial diseases and retinopathies
   Bart Leroy

7. Mitochondrial optic neuropathies
   Piero Barboni

8. The paradigm of mitochondrial optic neuropathies: naturally occurring compensatory strategies and treatment options
   Valerio Carelli

18:00 - 19:00 **Feedback on student presentations, awards presentation, summary of the course**

ABSTRACTS OF LECTURES

September, 24th

Overview of Clinical Ophthalmology for Basic Scientists

Antonio Ciardella
Sant'Orsola Malpighi Hospital, Bologna – Italy

This overview illustrates the use of clinical tools in the diagnosis of congenital retinal diseases. In particular it covers four hereditary conditions:

1. North Carolina Macular Dystrophy
2. Autosomal Recessive Bestrinopathies
3. Familial Amyloid Polineuropathy with Ocular Involvement
4. Enhanced S-Cone Syndrome

In each of the above diseases will be illustrated the clinical characteristics, and the utility of diagnostic techniques such as Fluorescein and Indocyanine Green Angiography (FAG / ICG), Optical Coherence Tomography (OCT), Fundus Autofluorescence (FAF) and Electrophysiology.

Overview of Basic Medical Genetics for Ophthalmologists

Bart P Leroy, MD, PhD

Dept of Ophthalmology & Ctr for Medical Genetics, Ghent University Hospital & Ghent University, Ghent, Belgium
&
Division of Ophthalmology & Center for Cellular and Molecular Therapeutics
The Children's Hospital of Philadelphia, University of Pennsylvania

Medical genetics is the young, dynamic and rapidly expanding medical specialty studying variability of phenotypes and genotypes of human disease.

Humans are thought to have between 20.000 and 22.000 genes. Of these, 293 genes (256 cloned) are now known to cause inherited retinal & optic nerve disease (RetNet @ http://www.sph.uth.tmc.edu/RetNet/).

The presentation will focus on explaining the current insights into genetics to an audience of ophthalmologists. Topics will include a review of Mendelian inheritance types and using pedigrees in the ophthalmic genetics clinic, a medical genetics glossary, mitosis and meiosis, current techniques in cytogenetics such as karyotyping, micro-array CGH, molecular mechanisms of disease such as different types of mutations and their respective effects such as point mutations, insertions and deletions, splice site mutations and their effects on protein formation, and methods of gene mapping. Finally, a brief review of current techniques of prenatal and pre-implantation genetic diagnosis will be mentioned.

Several excellent textbooks on medical genetics exist. Two of particular interest to course participants are:

1/ LB Jorde, JC Carey and MJ Bamshad: Medical Genetics, 4th Edition, Mosby Elsevier, 2010 (ISBN 978-0-323-05373-0)

2/ T Strachan & A Read: Human Molecular Genetics, 4th Edition, Garland Science, 2011 (ISBN 978-0-815-34149-9)

Two books that probably need to be in the library of anyone who interested in genetic eye disease are:
"Genetic Diseases of the Eye", 2nd Edition, Edited by EI Traboulsi, Oxford University Press; ISBN-10: 0195326148; ISBN-13: 978-0195326147; Publication Date: December 29, 2011

And a more recent superb textbook which provides concise chapters on most inherited retinal diseases is "Inherited Chorioretinal Dystrophies", Edited by Bernard Puech, Jean-Jacques De Laey & Graham E Holder, Springer-Verlag, Berlin Heidelberg, 2014, ISBN 978-3-540-69464-9

Stargardt disease, the Complex Simple Retinal Disorder

Rando Allikmets

Dept. of Ophthalmology, Columbia University, USA

The *ABCA4 (*then called *ABCR)* gene was cloned in 1997 as the causal gene for autosomal recessive Stargardt disease (STGD1, MIM 248200).[1] STGD1 is usually presents as a juvenile-onset macular dystrophy associated with rapid central visual impairment, progressive bilateral atrophy of the foveal retinal pigment epithelium, and the frequent appearance of yellowish flecks, defined as lipofuscin deposits, around the macula and/or in the central and near-peripheral areas of the retina.[2] Most STGD1 patients exhibit accumulation of lipofuscin throughout the retina, which is seen as a 'dark' choroid on fluorescein angiography[3] or, more recently, as an elevated autofluorescence in scanning laser ophthalmoscope (SLO) images.[4-6] Subsequently, *ABCA4* mutations were found to co-segregate with retinal dystrophies of substantially different phenotypes, such as autosomal recessive cone-rod dystrophy (arCRD)[7,8] and atypical autosomal recessive retinitis pigmentosa (arRP, RP19)[7,9,10] so, instead of using the term 'Stargardt disease', we now refer to all phenotypes caused by *ABCA4* mutations as '*ABCA4* disease'. Clinical heterogeneity of *ABCA4*-associated phenotypes further complicates the assessment of underlying genetic determinants for variable disease expression. An early disease model proposed a correlation between the continuum of disease phenotypes and residual ABCA4 activity/function,[7,11] where different combinations of "mild", "moderate", and "severe" *ABCA4* mutant alleles[12] were suggested to result in distinct phenotypes. The current model is more complicated, suggesting that some missense mutations, previously presumed more moderate than definitive null alleles (e.g., nonsense and frameshift mutations), could actually confer a dominant-acting gain-of-function.[13,14] Currently over 900 disease-associated *ABCA4* variants have been identified,[15] and the most frequent disease-associated *ABCA4* alleles, such as the p.G1961E variant, have each been described in only ~10% or less of patients.[16] The finding that 5% (1:20) of the general population carry a disease-associated *ABCA4* allele[17,18] has enormous implications for the amount of retinal pathology attributable to *ABCA4* variation.
Genetic analyses of *ABCA4*-associated retinal disease have been substantially advanced in recent years. New methods, such as direct sequencing of the entire genomic *ABCA4* locus,[19,20] have allowed detecting up to 80% of the disease-associated *ABCA4* alleles, including 2 (both) mutations in ~65-75% of patients. Of these ~80% are in the coding region and ~20% in introns, more than half of which

are outside of splice consensus sequences.[20-22] Of the rest, 1 mutation is detected in ~20% of patients while no disease-associated alleles are found in another 10% of screened patients with phenotypes compatible with the *ABCA4* disease. These data suggest that many (rare) disease-associated *ABCA4* alleles are yet to be identified and, most importantly, unequivocally confirmed by adequate functional analyses.

Other important advances in recent years have occurred in clinical description of ABCA4 disease which have become possible due to vast improvement in imaging methods, such as OCT, autofluorescence (AF), including quantitative AF,[4-6] and adaptive optics. As a result, ABCA4 diseases have been better categorized and disease progression quantitatively measured. More data have been acquired through advanced functional analyses.[23-25]

The presentation will summarize our current genetic, clinical and functional knowledge of ABCA4 disease and will suggest that a combination of advanced genetic screening coupled with advanced functional analyses of *ABCA4* alleles from both coding and non-coding sequences is necessary to unequivocally determine the *ABCA4*-associated disease load.[26]

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6. Burke TR, Duncker T, Woods RL, et al. Quantitative fundus autofluorescence in recessive Stargardt disease. *Invest Ophthalmol Vis Sci* 2014; **55**(5): 2841-52.
7. Cremers FP, van de Pol DJ, van Driel M, et al. Autosomal recessive retinitis pigmentosa and cone-rod dystrophy caused by splice site mutations in the Stargardt's disease gene ABCR. *Hum Mol Genet* 1998; **7**(3): 355-62.
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11. Lewis RA, Shroyer NF, Singh N, et al. Genotype/Phenotype analysis of a photoreceptor-specific ATP-binding cassette transporter gene, ABCR, in Stargardt disease. *American journal of human genetics* 1999; **64**(2): 422-34.
12. Maugeri A, van Driel MA, van de Pol DJ, et al. The 2588G-->C Mutation in the ABCR Gene Is a Mild Frequent Founder Mutation in the Western European Population and Allows the Classification of ABCR Mutations in Patients with Stargardt Disease. *American journal of human genetics* 1999; **64**(4): 1024-35.
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14. Zhang N, Tsybovsky Y, Kolesnikov AV, et al. Protein misfolding and the pathogenesis of ABCA4-associated retinal degenerations. *Hum Mol Genet* 2015; **24**(11): 3220-37.

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16. Burke TR, Fishman GA, Zernant J, et al. Retinal phenotypes in patients homozygous for the G1961E mutation in the ABCA4 gene. *Invest Ophthalmol Vis Sci* 2012; **53**(8): 4458-67.
17. Jaakson K, Zernant J, Kulm M, et al. Genotyping microarray (gene chip) for the ABCR (ABCA4) gene. *Human mutation* 2003; **22**(5): 395-403.
18. Yatsenko AN, Shroyer NF, Lewis RA, Lupski JR. Late-onset Stargardt disease is associated with missense mutations that map outside known functional regions of ABCR (ABCA4). *Human genetics* 2001; **108**(4): 346-55.
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21. Bauwens M, De Zaeytijd J, Weisschuh N, et al. An augmented ABCA4 screen targeting noncoding regions reveals a deep intronic founder variant in Belgian Stargardt patients. *Human mutation* 2015; **36**(1): 39-42.
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Overview of Inherited Corneal Disorders

Graeme Black
University of Manchester
and
Central Manchester University Hospitals NHS Foundation Trust, Manchester Academic Health Sciences Centre (MAHSC), St. Mary's Hospital

The cornea is, with the lens, the major refractive structure of the anterior segment of eye. It comprises a non-keratinised epithelium, a collagenous stroma and a non-dividing endothelial monolayer. This lecture will first give an overview of a range of inherited corneal abnormalities including:
Corneal dystrophies – a range of bilateral, progressive, non-inflammatory disorders. (See ic3d classification)
http://www.corneasociety.org/sites/default/files/publications/ic3d_class_cornealdystrophies.pdf
These may be classified by site (epithelial / stromal / endothelial) or by molecular defect.
In the first part of this talk the discovery of:

i) Keratin mutations (Krt3/12) which underlie Meesmann epithelial dystrophy (MECD)
ii) TGFBI, which underlies a range of epithelial and stromal disorders including granular lattice and Avellino corneal dystrophies.
iii) COL8A2 underlying endothelial dystrophies

Corneal clouding during childhood is another important group of disorders which may be caused by congenital glaucoma, early-onset dystrophic processed (endothelial dystrophy) and may also be associated with systemic abnormalities such as lysosomal storage disorders.
Corneal thinning is another important group of disorders. Brittle cornea syndrome is an important condition to recognise that may be associated with corneal fragility and blindness. The discovery of genes underlying Brittle Cornea syndrome (ZNF469, PRDM5) and their relationship to common disorders such as ketatoconus will be discussed.
The cornea is an attractive site for attempting molecular therapeutics as it is accessible, small, non-vascular and immune-privileged. Therapeutic approaches that will be discussed include the use of siRNA and CRISPR/Cas9 technologies in the treatment of MECD.

Molecular basis of non- syndromic and syndromic retinal and vitreoretinal diseases

Wolfgang Berger
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Monogenic diseases of the retina and vitreous affect approximately 1 in 2000 individuals, or more than 3-4 million people worldwide. Consequences for affected individuals are variable and can range from legal blindness in the most severe forms of retinal degenerations (Leber congenital amaurosis, LCA) to less severe or rather mild retinal dysfunctions (night blindness, achromatopsia). The diseases can be categorized in four major groups: (i) rod dominated diseases, (ii) cone dominated diseases, (iii) generalized retinal degenerations (affecting both photoreceptor cell types, rods and cones), and finally (iv) exudative as well as erosive vitreoretinopathies. The disease classification also considers whether the ocular phenotype is associated with pathologies of other tissues (syndromic forms) or only affects the retina, retinal pigment epithelium and the vitreous body (non-syndromic forms). In addition, the mode of inheritance is also used as one characteristic feature of the different disease phenotypes in order to categorize them.

For most of them, no treatment can be offered. In the past 20-25 years, knowledge about the molecular basis of retinal diseases has tremendously progressed and evidence for the contribution of genetic factors but also environmental circumstances is continuously accumulating. After a time period that was mainly characterized by the identification of genes and disease causing mutations for the monogenic retinal and vitreoretinal traits in families, we now have entered an era where not only monogenetic (classic Mendelian) but also multifactorial diseases are in the interest of clinical, genetic and basic research. Still, a reliable molecular diagnosis is possible for only half of the affected individuals or families with monogenic forms of retinal diseases. In addition, the predictive value of a mutation or risk allele for multifactorial disorders is problematic since the phenotypic and/or symptomatic consequences are highly variable. Nevertheless, the knowledge about the molecular mechanisms has also improved diagnostic assessment of patients by genetic testing, in particular by applying next generation sequencing (NGS). It is the ultimate goal to better understand the molecular etiology of these diseases and to develop approaches for therapeutic interventions.

As phenotypes do not always correlate with the respective genotypes, it is of utmost importance that clinicians, geneticists, counsellors, diagnostic laboratories and basic researchers understand the relationships between phenotypic manifestations and specific genes, as well as mutations and pathophysiologic mechanisms.

Reference:

Berger W, Kloeckener-Gruissem B, and Neidhardt J (2010) The molecular basis of human retinal and vitreoretinal diseases. ***Prog Retin Eye Res*** 29:335-375

Introduction to Next-Generation Sequencing for Eye Diseases

Susanne Roosing
Dept. of Human Genetics, Radboud University Medical Center, Nijmegen, The Netherlands

For many years Sanger sequencing has been the golden standard in DNA diagnostic laboratories. However, implementing Sanger sequence gene tests for all known monogenic diseases in a single laboratory is impossible. Moreover, Sanger sequencing is impossible when the causative gene of the genetic diseases is still unknown. Genetically heterogeneous disorders such as inherited retinal diseases (IRD) add another level of complexity to clinical diagnosis and have therefore been difficult to diagnose in a routine DNA diagnostic setting up till now. Available methods like the ASPER primer extension chips were designed to detect known variants, they however fail when a patient has causative mutations that have not been described in literature. Therefore, in many patient samples remained genetically undiagnosed. Next generation sequencing (NGS) opens the way to high throughput analysis of either targeted genomic regions or even the whole exome or genome and allowed us for the first time to simultaneously sequence all known genes that are involved in IRD.

In 2012 a targeted NGS study was developed for all known inherited retinal disease genes, and applied to 100 patients with autosomal recessive or isolated retinitis pigmentosa, a specific IRD. Mutations were identified in arRP genes (n=27), X-linked RP genes (n=3), and autosomal dominant RP genes (n=6). In at least 3 families *de novo* mutations were found. Taking into consideration that the patients were derived from a larger cohort that was prescreened for mutations in selected retinal dystrophy genes, this approach could find disease-causing mutations in ~50% of patients with isolated or autosomal recessive RP.
But, several disadvantages are linked to targeted sequencing. The design of such a targeted approach is very work intensive and still novel IRD genes are published on a regular basis, therefore the panel must be adjusted frequently. Furthermore, the panels are disease-specific and sometimes it is difficult to correctly diagnose a patient.
Therefore a generic workflow for diagnostic exome sequencing has been developed, with a focus on heterogeneous disorders such as hereditary blindness, but also hereditary deafness, mitochondrial diseases, movement disorders, cancer, etc. In ~55% of a heterogeneous group of IRD patients the genetic cause of the IRD could be determined. Taking into consideration that the patients were derived from a larger cohort that was prescreened for mutations in selected retinal dystrophy genes, this approach could find disease-causing mutations in ~65% of patients with IRD.

During my presentation I will present and compare both targeted and exome sequencing for blindness in a diagnostic setting. Topics that will be discussed include amongst others coverage, quality control, diagnostic yield, and incidental findings. I will demonstrate that exome sequencing has become a robust approach for the identification of genetic variation and can be implemented in a diagnostic setting, even for genetically heterogeneous diseases such as blindness. Also the exome-successors whole genome sequencing and PacBio sequencing will be discussed.

Literature:
Diagnostic exome sequencing in 266 Dutch patients with visual impairment.
Haer-Wigman L, van Zelst-Stams WA, Pfundt R, van den Born LI, Klaver CC, Verheij JB, Hoyng CB, Breuning MH, Boon CJ, Kievit AJ, Verhoeven VJ, Pott JW, Sallevelt SC, van Hagen JM, Plomp AS, Kroes HY, Lelieveld SH, Hehir-Kwa JY, Castelein S, Nelen M, Scheffer H, Lugtenberg D, Cremers FP, Hoefsloot L, Yntema HG.
Eur J Hum Genet. 2017 May;25(5):591-599

Next-generation genetic testing for retinitis pigmentosa.
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A post-hoc comparison of the utility of Sanger sequencing and exome sequencing for the diagnosis of heterogeneous diseases
Kornelia Neveling, Ilse Feenstra, Christian Gilissen, Lies H. Hoefsloot, Erik-Jan Kamsteeg, Arjen R. Mensenkamp, Richard J.T. Rodenburg, Helger G. Yntema, Liesbeth Spruijt, Sascha Vermeer, Tuula Rinne, Koen L. van Gassen, Danielle Bodmer, Dorien Lugtenberg, Rick de Reuver, Wendy Buijsman, Ronny C. Derks, Nienke Wieskamp, Bert van den Heuvel, Marjolijn J.L. Ligtenberg, Hannie Kremer, David A. Koolen, Bart P.C. van de Warrenburg, Frans P.M. Cremers, Carlo L.M. Marcelis, Jan A.M. Smeitink, Saskia B. Wortmann, Wendy A.G. van Zelst-Stams, Joris A. Veltman, Han G. Brunner, Hans Scheffer and Marcel R. Nelen
Hum Mutat. 2013 Dec;34(12):1721-6.

Genetic Counselling in Inherited Eye Disease

Ms Georgina Hall and Dr Marco Seri
Central Manchester and Manchester Children's Foundation Trust, Manchester, UK
Medical Genetics Unit, Sant'Orsola Malpighi Hospital, University of Bologna, Italy

In this workshop, we will discuss the aims of genetic counselling for individuals and families with inherited eye disease and explore services available. Using three key themes in genetic counselling 1) family impact, 2) predictive testing and 3) new genetic technology, we will present cases illustrating the counselling challenges. Working in small groups, participants will have the opportunity to work with real cases to debate counselling approaches and ethical dilemmas. Participants are welcome to bring their own cases and questions relating to these three themes and these will be incorporated if time permits.
Learning objectives:

1. To understand the aims of genetic counselling and the ways services are provided.
2. To appreciate the way genetic eye disease can impact on families and the counselling issues and ethical dilemmas

September, 25th

Genetics of Retinitis Pigmentosa, Leber Congenital Amaurosis and Congenital Stationary Night Blindness

Bart P Leroy, MD, PhD

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Division of Ophthalmology & Center for Cellular and Molecular Therapeutics
The Children's Hospital of Philadelphia, University of Pennsylvania

Introduction:
Retinitis pigmentosa (RP) is a retinal dystrophy of the rod-cone type with early night blindness, followed by progressive constriction of the visual fields and eventually some degree of loss of central vision.
Leber congenital amaurosis (LCA) is a genetically and clinically heterogenous hereditary retinal disorder causing profound visual loss, nystagmus, poorly reactive pupils and a markedly diminished electroretinogram (ERG) due to the loss of photoreceptor function. Theodor Leber first described the condition in 1869 as a severe form of retinitis pigmentosa presenting in infancy or early childhood, with the absence of photoreceptor function.
Congenital stationary night blindness (CSNB) is, just like RP and LCA, a group of conditions. The common symptom is either a total, or at least some degree of night blindness, which is of congenital onset and is stationary over time. The aspect of the electroretinography in combination with the clinical presentation is paramount to make the diagnosis, and classify the subtype.
Methods:
An overview of the current status of knowledge regarding phenotypes and genotypes of RP, LCA and CSNB will be presented.
Results:
RP can be inherited as an autosomal dominant, an autosomal recessive or an X-linked trait. There are currently 22 genes known for ADRP, 36 for ARRP and 2 for XLRP. The underlying molecular mechanisms are very different, with the spectrum of genes involved encoding proteins ranging from phototransduction proteins to pre-mRNA splicing factors.
The molecular genetics of LCA has also been studied intensely over the last decade. All 24 genes so far identified, *GUCY2D*, *RPE65*, *CRX*, *AIPL1*, *CRB1*, *RPGRIP1*, *MERTK*, *RDH12*, *IMPDH1*, *TULP1*, *CEP290*, *LCA5*, *SPATA7*, *OTX2*, *IQCB1*, *PDE6G*, *KCNJ13*, *NMNAT1*, *RD3*, *DTHD1*, *CAPB4*, *GDF6*, *IFT140* & *PRPH2* have very different functions in the retina. Together they account for about 70% of all patients. It is also becoming increasingly clear that particular phenotypes can sometimes be attributed to specific genotypes.
Mutations in 13 different genes have been identified as the cause of autosomal dominant, autosomal recessive or X-linked CSNB. Mutations in these genes account for the large majority of CSNB patients.
Conclusion:
With the discovery a large number of genes to date, still less than half of RP patients, but a majority of LCA and CSNB patients have been molecularly accounted for. In addition, some genotype-phenotype correlations seem to exist, all together helping to increase knowledge about the pathogenesis of these conditions. This is essential in this day and age of emerging treatment strategies.
Reference:
"RetNet, http://www.sph.uth.tmc.edu/RetNet/"

Stem Cells in Eye Disease -Cell replacement therapies for retinal disease

Jane C Sowden
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One in 3000 people have an inherited retinal disease caused by mutations in any one of more than 200 different genes. More than 3.2 million people are currently blind from age-related macular degeneration, and this number is set to rise in aging populations. The scale of untreatable blindness involving the loss of photoreceptor cells provides a strong impetus for the development of stem cell therapies. The success of gene therapy relies on the delivery of new functional genes to cells that lack such genes and is therefore dependent upon cell survival and needs to be tailored to each gene. By contrast stem cell therapies offer a complementary approach that may be applicable to a broad range of retinal degenerations. Pluripotent stem cell lines offer the potential to generate unlimited quantities of new retinal cells for transplantation. Both embryonic stem cell lines and induced pluripotent stem cells (IPSC; derived from an individual's somatic cells) provide a renewable source of human cells. Remarkably, recent studies have shown that it is possible to generate three-dimensional synthetic retinal structures from cultures of pluripotent stem cells. As well as providing replacement cells, patient-derived IPSC provide model systems to study disease pathways and identify possible therapeutic interventions to prevent retinal cell loss. Clinical trials are evaluating retinal pigment epithelium cell transplantation as an approach to preserve photoreceptor cells. Other pre-clinical studies are developing photoreceptor cell transplantation to replace those cells lost through disease. Our proof of concept studies showed that transplantation of rod photoreceptors isolated from the postnatal mouse retina restored some visual function in a mouse genetic model of night-blindness supporting the feasibility of photoreceptor transplantation. These promising steps and new insights for the future development of retinal stem cell therapy will be discussed.

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Genetics Basis of Age-Related Macular Degeneration (AMD)

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A major goal of human genetic research today is to determine the extent that genetic variation plays in complex disease. Central to this understanding is the identification of informative variants and determination of their biological manifestations. While progress has been made for some complex diseases, considerable discussion remains regarding the role of common versus rare variants, deletions and insertions, epigenetic effects and gene-gene and gene-environment interactions for most common disorders[1].

Remarkable advances in deciphering one's genetic susceptibility to age-related macular degeneration (AMD) -- a late-onset complex disorder that affects nearly one-third of the population over the age of 75 -- have been made over the past twelve years. A breakthrough in our understanding of this disease started with the discovery in 2005 that common variants in the complement factor H gene (*CFH*) on human chromosome 1q32, a gene that encodes the major regulator of the complement alternative pathway, are significantly associated with the disease[2-5]. This was followed by the identification of an equally significant association of two tightly linked genes (*ARMS2* and *HTRA1*) on human chromosome 10q26[6,7] and by the observation of an association with two additional complement regulators on chromosome 6p21, complement factor B and component 2 (*CFB/C2*)[8]. Variation at these three loci defines a major fraction of the disease burden, making AMD one of the most well genetically defined complex traits.

Based on all available genetic data, we can suggest hypotheses related to the role of inflammation and aberrant complement activation in the development and progression of AMD[2, 11]. There is strong support for the concept that a major subset of AMD is initiated by aberrant control of the complement alternative pathway likely due to dysfunction of CFH[9]. Once the pathway is triggered, inflammation is prolonged in individuals with predetermined genetic susceptibility (*i.e.*, a specific combination of *CFH, ARMS2/HTRA1*, and *CFB/C2* haplotypes). The specific agent(s) that trigger the complement cascade at the level of the RPE-choroid interface are being identified, including infections and factors increasing oxidative stress. This sustained, chronic inflammation leads to drusen formation, a hallmark feature of early AMD. Current data suggest that disease progression into advanced forms may be largely driven by genetic variation at the 10q26 locus[10] and additional genetic and environmental factors. Another possibility is that the complement pathway ad the 10q26 locus act largely as independent determinants of the disease we call AMD and that the disorder is actually a collection of several different diseases we call by the same name.

The presentation will summarize the current knowledge of the role of all major and more minor AMD-associated genetic loci[12, 13] and discuss the clinical implications of this information. The presentation is geared for clinician-scientists and geneticists to explain the how we can use the genetic information for the patient's benefit.

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Overview of Developmental Eye Anomalies

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Ocular developmental disorders span a wide spectrum and comprise one of the commonest groups of birth defects.
This overview will examine a number of these groups including:

i) Microphthalmia / Anophthalmia, including Coloboma or optic fissure closure defects, both Isolated and syndromic.
ii) Congenital Cataract, both Isolated and Syndromic.
iii) Anterior Segment Dysgenesis including Congenital glaucoma, Peters /Rieger anomaly / Aniridia.

For each of these groups a small number of key examples will be discussed to understand the underlying themes. These will include; X-linked microphthalmia (Lenz) and Oculofaciocardiodental syndrome; microphthalmia and SOX2/OTX2; congenital cataract and lens-specific gene mutation; aniridia and PAX6, Rieger syndrome and PITX2.
The talk will examine the value of novel technological approaches to diagnosis and the power of next generation sequencing approaches in the diagnosis of developmental ocular disease.

Retinal Ciliopathies: Diverse Phenotyopes with Overlapping Genetic Structure

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Despite remarkable progress in the identification of mutations that drive genetic disorders, progress in understanding the effect of genetic background on the penetrance and expressivity of causal alleles has been modest, in part because of the methodological challenges in identifying genetic modifiers. Nonetheless, the progressive discovery of modifier alleles has improved both our interpretative ability and our analytical tools to dissect such phenomena. In this lecture, we analyze the genetic properties and behaviors of modifiers as derived from studies in patient populations and model organisms and we highlight conceptual and technological tools used to overcome some of the challenges inherent in modifier mapping and cloning. Finally, we discuss how the identification of these modifiers has facilitated the elucidation of biological pathways and holds the potential to improve the clinical predictive value of primary causal mutations and to develop novel drug targets.

September, 26th

Genetics of Glaucoma

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Glaucoma is the leading cause of untreatable blindness worldwide, affecting around 1 in 40 people over the age of 40. It is a neurodegenerative disease affecting the optic nerve and resulting in death of retinal ganglion cells and irreversible visual field loss. Glaucoma is both clinically and genetically heterogeneous and the pathogenic mechanisms are not well understood. It can be congenital, or with juvenile or adult onset. Primary open-angle glaucoma (POAG) is the most common form of glaucoma and shows a significant heritability with a relatives of affected individuals having a 5-10 times increased lifetime risk, although the majority of forms do not show clear Mendelian patterns of inheritance. Genetic linkage studies in rare families showing Mendelian patterns of inheritance have identified POAG genetic loci (GLC1A-Q) and loci for congenital glaucoma causing monogenic disease. Notably however, very few of these genes (MYOC, OPTN) have been robustly associated with POAG in the general population and identified gene mutations account for < 10% of cases overall. To find additional genes contributing to glaucoma pathology genome wide association studies (GWAS) have been conducted comparing the frequency of common genetic variations (single nucleotide polymorphisms; SNPs) between glaucoma cases and control populations. These studies have identified a set of genetic risk factors (SNPs located near new genes) that confer modest risk for glaucoma (e.g. CAV1 & CAV2, TMOC1, CDKN2BAS; odd ratios 1.3-1.7) within the studied populations. Raised intraocular pressure (IOP) is one of the strongest known risk factors for glaucoma; other risk factors include reduced central corneal thickness (CCT) and optic nerve cupping measured by enlarged cup disc ratio (CDR). These quantitative traits related to glaucoma, referred to as endophenotypes, are heritable and have also been used in GWAS studies to further dissect the genetic components leading to disease susceptibility. As yet the causative variants that alter gene function and lead to ocular tissue changes and retinal ganglion cell death remain to be identified for the genetic risk factors identified in GWAS studies. These studies are nevertheless providing vital tools to unravel the molecular mechanisms and pathophysiology underlying glaucoma complexity. The future goal is to use whole genome analyses to develop clinically useful genetic tests that identify individuals at risk of developing glaucoma so that early treatment (by lowering IOP) can prevent visual loss, in combination with the development of novel therapeutic strategies based on new knowledge of the molecular basis of disease pathways.

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Gene therapy for recessive and dominant eye disorders

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Inherited retinopathies (IR) are common untreatable blinding conditions. Most of them are inherited as monogenic disorders, due to mutations in genes expressed in retinal photoreceptors (PR) and in retinal pigment epithelium (RPE). The retina's compatibility with gene transfer has made transduction of different retinal cell layers in small and large animal models via viral and non-viral vectors possible. To date, recombinant vectors based on the adeno-associated virus (AAV) represent the most promising tool for retinal gene therapy, given their ability to efficiently deliver therapeutic genes to both PR and RPE and their excellent safety and efficacy profiles in humans. The ongoing identification of novel AAV serotypes as well as modifications of existing ones based either on rational design or directed evolution have generated vector variants with improved transduction properties. Dozens of promising proofs of concept have been obtained in IR animal models with AAV, and some of them have been relayed to clinical trials. However, AAVs' limited cargo capacity has prevented application of the viral vector to treatments requiring transfer of genes with a coding sequence larger than 5 kb. Strategies to overcome this limitation will also be discussed.

The Role of Non-Coding RNAs in Eye Development and Function

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Non-coding transcripts (ncRNA) represent functional RNA molecules that do not encode for proteins. It is now widely accepted that ncRNAs are endowed with a previously unrecognized role in many biological processes, mainly through regulation of gene expression. Among ncRNAs, microRNAs (miRNAs) have received the most attention by far: plants and animals contain hundreds of distinct miRNA genes, and these in turn help to regulate the expression of an even larger number of mRNAs. MicroRNAs (miRNAs) are a class of small, endogenous RNAs that negatively regulate gene expression post-transcriptionally by binding to target sites in the 3' untranslated region (UTR) of messenger RNAs (1). Although they have been found to regulate developmental and physiological processes in several organs and tissues (2), their role in eye function is still largely unknown. To begin understanding their eye-related function in mammals, we first generated a comprehensive expression atlas of miRNAs significantly expressed in the mouse eye by means of RNA *in situ* hybridization (ISH) (3). We then reconstructed the architecture of the entire human retina miRNome by Next Generation Sequencing procedures (4). The latter analysis allowed us to identify a number of putative novel miRNAs that seem to be preferentially expressed in the human retina. In parallel, we have started a characterization of the functional role of miR-204, which is among the most highly expressed miRNAs in several eye structures including retina, retinal pigment epithelium, and lens. We demonstrated, by means of both *in vitro* and *in vivo* studies, that miR-204 regulates multiple aspects of eye development and function and identified some of the most relevant target genes (5-6). Finally, we found that a point mutation in miR-204 is responsible for an autosomal dominantly inherited form of retinal dystrophy and bilateral coloboma through a gain-of-function mechanism (7). These data provide the first example of a microRNA with a pathogenic role in inherited retinal dystrophies.

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Modifier Genes and Digenic Inheritance in Retinal Diseases

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Inherited retinal diseases display a high degree of clinical and genetic heterogeneity. DNA variants can have an effect on the mRNA expression level, stability, or splicing, or on protein structure, stability, and function. Variations in single genes however do not account for all clinical variation as both within families as between families with identical mutations, huge differences are seen in disease onset and progression. Apparently, other genetic or non-genetic factors play a role in this variation. When these factors play a minor role, they are considered to be modifiers of disease expression. In digenic inheritance, variants in two genes are necessary to elicit a clinical phenotype. In this lecture, five examples will illustrate allelic and non-allelic modifiers, as well as digenic inheritance, in human inherited retinal diseases.

Heterozygous PRPH2/Peripherin and ROM-1 mutations result in digenic RP

Heterozygous mutations in *PRPH2/Peripherin* generally are associated with a wide spectrum of autosomal dominant retinal dystrophies that can affect both the rod and cone systems. Variants in the *ROM-1* gene have not yet been associated with monogenic retinal disease. Kajiwara et al. (1994) identified non-allelic heterozygous variants in *PRPH2* (p.Leu185Pro) and *ROM1* (protein-truncating mutations) in persons with RP. p.Leu185Pro-mutant PRPH2 is unable to form PRPH2 homotetramers, which, together with ROM-1, are needed for higher-order disulfide-linked oligomer formation (Goldberg et al. 1995; Loewen et al. 2001). The levels of these oligomers is critical for stable photoreceptor disc formation.

PRPF31 and allelic modifier.
Mutations in the pre-mRNA splicing gene *PRPF31* were found in families with autosomal dominant RP (Vithana et al. 2001). However, many mutation-carriers were found to be asymptomatic. Haplotype and mRNA analyses revealed that the mRNA expression levels of the wildtype *PRPF31* alleles determined the clinical outcome. Asymptomatic carriers of *PRPF31* mutations showed relatively high mRNA expression of the normal allele, whereas RP patients showed low levels of mRNA expression (Vithana et al. 2003). Non-penetrance is frequently encountered in autosomal dominant diseases, and in case of a haplo-insufficiency disease model, the mRNA expression level of the normal allele in many cases may explain the non-penetrance.

BBS1: allelic and non-allelic modifiers
Mutations in the *BBS1* gene cause a large proportion of Bardet-Biedl syndrome (BBS), the most frequent of which, p.M390R, is found in ~20% of all BBS patients. In a family with two BBS siblings and homozygous p.M390R mutations, Beales et al. (2003) found that the unaffected mother was heterozygous for this variant and that the unaffected father was homozygous for this variant. Badano et al. (2006) found a genetic modifier in *MGC1203* (*BBSIP1*) in both affected siblings but not in the unaffected father. This variant, c.430C>T, had a moderate effect on the splicing of intron 2. We identified a homozygous p.M390R variant in 11 of 2,256 probands with RP and in one of 1,724 ethnically matched controls (A. Estrada-Cuzcano et al. 2012). Hardy-Weinberg calculations suggest that the p.M390R variant 'behaves' as a regular autosomal recessive mutation according to Mendelian inheritance rules. It is hypothesized that in some healthy individuals with homozygous p.M390R variants, one or both mutation-carrying alleles are highly expressed and thereby compensate for the hypomorphic detrimental effect of p.M390R.

A similar example of non-penetrance was found for a frequent *NMNAT1* variant (p.E257K), which was found to be associated with Leber congenital amaurosis. Based on its frequent carriership in control individuals (1/500 Europeans), it should explain 5% of all LCA cases when present in a homozygous state. It is found in ~5% of LCA cases in a compound heterozygous manner, but only in 0.1% of LCA cases in a homozygous state. This 50-fold difference can only be explained by assuming that it is a hypomorphic allele that only in exceptional cases is associated with LCA in a homozygous state (Siemiatkowska et al. 2014, and refs therein).

MERTK variant as a modifier for CEP290-associated retinal dystrophy
Two siblings of a Dutch family showed early-onset severe retinal dystrophy (EOSRD) due to compound heterozygous hypomorphic *CEP290* mutations. A cousin of these siblings carried the same *CEP290* variants, but shows a more severe phenotype, LCA. A second cousin of these patients showed a classical RP phenotype due to a homozygous *MERTK* stopmutation (Littink et al. 2010). Segregation analysis revealed that the LCA patient, but not the EOSRD patients, carried the *MERTK* stopmutation heterozygously. The CEP290 and MERTK proteins act in different, but functionally related systems. CEP290 is a critical component of the connecting cilium which transports molecules between the inner and outer segments and thereby is important for the creation of new photoreceptor discs. MERTK is involved in the phagocytosis of photoreceptor outer segment disc, 10% of which are shed each night. It can be hypothesized that defects on both sides of these discs have a cumulative effect.

Putative digenic inheritance of C2orf71 and RP1L1 variants in syndromic atypical RP
In an isolated Danish person with atypical RP, congenital hearing loss and cerebellar atrophy, whole exome sequencing revealed heterozygous null mutations in *C2orf71* and *RP1L1*. Biallelic *C2orf71* mutations and biallelic *RP1L1* mutations have previously been associated with autosomal recessive RP. We hypothesized that this combination of heterozygous variants results in the syndromic RP phenotype. Using zebrafish KD studies we showed additive eye and cerebellar defects which support this hypothesis (Liu et al. 2016).

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Genetics of Mitochondrial Diseases & Retinopathies

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Mitochondria are the powerhouses of the cell, producing ATP as cellular fuel via oxidative phosphorylation. This system consists of 5 multiprotein complexes involving more than 100 polypeptides. Only 13 of these are encoded for by mitochondrial DNA (mtDNA), with the rest encoded by nuclear genes. The human mtDNA consists of 16 kb of circular ds DNA with a total of 37 genes. Apart from the 13 that endode protein subunits of the oxidative phosphorylation pathway, 22 encode mt tRNA's and 2 code for rRNAs.

Mitochondrial diseases are a heterogeneous group of disorders of energy metabolism, which are present at all ages and in which the eyes are frequently affected. When the eyes are involved, the retina, optic nerve and extra-ocular muscles are most frequently affected.

Mitochondrial conditions, which affect the retina include:
Neurogenic muscle weakness, ataxia and retinitis pigmentosa (NARP)
Leigh syndrome
Mitochondrial encephalopathy, lactic acidosis & stroke-like episodes (MELAS)
Maternally inherited diabetes & deafness with maculopathy (MIDD)
Myoclonic epilepsy & ragged red fibers (MERRF)
Kearns-Sayre syndrome (KSS)
Mitochondrial myopathy.

An overview of these conditions will be provided, illustrating their most frequent associated signs and symptoms. Tests essential to make a diagnosis, as well as some caveats will be discussed.

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OMIM

Mitochondrial Optic Neuropathies

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Mitochondrial optic neuropathies are characterized by extreme selectivity of tissue expression, as the retinal ganglion cells and the optic nerve are the only affected sites. Another common feature, and a hallmark of mitochondrial optic neuropathies, is the early and preferential involvement of the small fibers in the papillomacular bundle that serves central vision. Two diseases with both overlapping features and important differences belong to this category, Leber's Hereditary Optic Neuropathy (LHON) and dominant optic atrophy (DOA). LHON is due to mtDNA point mutations and obeys the rules of mitochondrial genetics, whereas DOA is due to nuclear gene mutations and is transmitted as an autosomal dominant trait. Although the two disorders have different genetic etiologies and different progressions (one is subacute, the other slowly progressive), both show variable penetrance, which is not easily explained. Moreover, from a clinical point of view, we summarize the mechanisms and recent developments of optical coherence tomography (OCT) and its practical uses in mitochondrial optic neuropathies. The application of OCT in syndromic and not syndromic mitochondrial neuropathies are reviewed.

The Paradigm of Mitochondrial Optic Neuropathies: Naturally Occurring Compensatory Strategies and Treatment Options

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Mitochondrial optic neuropathies, the two most prevalent being Leber's hereditary optic neuropathy (LHON) and Dominant optic atrophy (DOA), are now reasonably well known disorders for their genetics, clinical features and natural history. This and the mono-symptomatic nature of both diseases make them particularly suitable for testing therapeutic options. Another point of interest is the reduced penetrance, particularly evident in LHON, which if understood may provide valuable information on the relevant compensatory mechanisms that protect from developing the clinical symptoms, in presence of the genetic mutation.

We recently focused on two unsolved related issues concerning LHON, i.e. male prevalence and incomplete penetrance. In both cases, we reached evidence that efficient activation of mitochondrial biogenesis plays a key role in protecting mutation carriers from developing the optic neuropathy. Gender bias relates to the role of estrogens in activating a successful compensatory increase of mitochondrial biogenesis in females. The efficiency of mitochondrial biogenesis also distinguishes affected of both genders from the unaffected mutation carriers, who on average display the highest mtDNA copy number. Thus, dissecting the details of the signaling pathways and the genetic background underlying efficient mitochondrial biogenesis will provide key clues on the successful occurrence of spontaneous compensatory mechanisms preserving from blindness in LHON.

These findings suggest to exploiting any strategy that safely increases mitochondrial biogenesis as possible therapy for LHON and may be for other mitochondrial disorders. While this may be the approach to prevent LHON, currently patients continue to become affected in LHON families and the disease process progresses over time in DOA. We recently pursued the therapeutic option of using antioxidant molecules that may also help in bypassing complex I dysfunction for both LHON and DOA, treating in open label trials patients with idebenone or EPI-743. In both cases, this approach

may help in a subgroup of responding patients, with the necessary caution and the need to consolidate these preliminary indications with well-designed controlled trials. We also believe that other options should be adopted, in particular for the acute phase of LHON, and there is currently the first ongoing gene therapy trial in LHON. Overall, there is a change in the paradigm, shifting from the untreatable condition to the availability of some treatments.

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5th Course in Eye Genetics

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