EUROPEAN SOCIETY OF HUMAN GENETICS

European School of Genetic Medicine

4th Course in

Eye Genetics

Bertinoro, Italy, September 27-29, 2015

Bertinoro 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).

EUROPEAN SCHOOL OF GENETIC MEDICINE

ALMA MATER STUDIORUM
UNIVERSITÀ DI BOLOGNA

CENTRO RESIDENZIALE UNIVERSITARIO Bertinoro

4th Course in

Eye Genetics

Bertinoro, Italy, September 27-29, 2015

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4TH COURSE IN EYE GENETICS

Bertinoro University Residential Centre
Bertinoro, Italy, September 27-29, 2015

Arrival day: Saturday, September 26th

September 27

8:30 - 8:40 **Welcome**

8:40 - 9:10 History of Medical Genetics
Giovanni Romeo

9:15 - 10:00 **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

10:05 - 11:35 **2 talks (40 min + 5 min discussion)**

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

4. Overview of inherited corneal disorders
Graeme Black

11:35 - 12:00 **Break**

12:00 - 13:30 **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
Lonneke Haer-Wigman

13:30 - 14:30 **Lunch**

14:30 - 16:15 **3 parallel workshops**

Garrison room

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

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

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

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

16:45 - 18:30 **3 parallel workshops**

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

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

Computer room
WS3 Disease-causing mutations: finding, interpretation, nomenclature (**Berger & Allikmets**)

September 28

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 AMD
   Rando Allikmets

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

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

4. AAV vectors for gene retinal therapy
   Alberto Auricchio

5. Modifier genes and digenic inheritance in retinal diseases
   Frans Cremers

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

14:15 - 16:00 **3 parallel workshops**

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

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

Computer room

WS3 Disease-causing mutations: finding, interpretation, nomenclature (**Berger & Allikmets**)

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

16:30 - 18:15 **2 parallel workshops**

Jacopo da Bertinoro Room
WS1 Final preparation for student presentations and selection of 10-12 cases for presentation (**Black & Leroy**)

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

September 29

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. Overview of developmental eye anomalies
   Graeme Black

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

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**

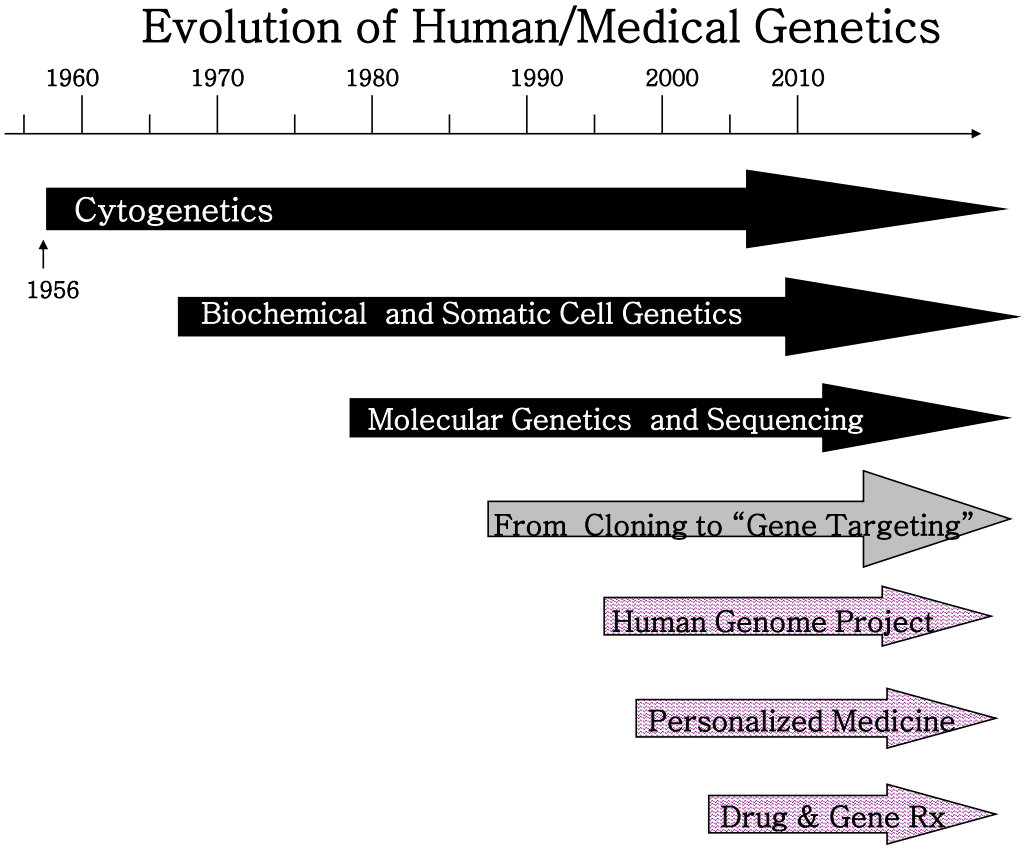
ABSTRACTS OF LECTURES

September, 27th

History of Medical Genetics

Giovanni Romeo
University of Bologna, European School of Genetic Medicine, Bologna, Italy

The main steps in the evolution of contemporary Human/Medical Genetics will be summarized and each step will be linked to the professional history of a scientist or clinician who left a mark in our field. Along with the research blooming in this period (see figure), some educational initiatives were developed in America and Europe, like the Short Course in Medical Genetics started by V.A. McKusick in Bar Harbor, Maine-USA, in 1960, the European School of Genetic Medicine started in Sestri Levante, Italy, in 1988 and the Latin American School of Human and Medical Genetics started by Roberto Giugliani in Caxias do Sul, Brazil, in 2005. During the same years these courses and schools trained thousands of young geneticists coming from all over the world and contributed to the transition from genetic to genomic medicine.



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 25.000 genes. Of these, 278 genes (238 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)

A book that needs to be in the library of anyone who interested in genetic eye disease is:

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

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 75% are in the coding region and 25% in introns, more than half of which are outside of splice consensus sequences. Of the rest, 1 mutation is detected in ~20% of patients while no disease-associated alleles are found in another 15% 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.[21-22]

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.

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10. Shroyer NF, Lewis RA, Yatsenko AN, Lupski JR. Null missense ABCR (ABCA4) mutations in a family with stargardt disease and retinitis pigmentosa. *Invest Ophthalmol Vis Sci* 2001; **42**(12): 2757-61.
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. *Am J Hum Genet* 1999; **64**(2): 422-34.
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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). *Hum Genet* 2001; **108**(4): 346-55.
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Overview of Inherited Corneal Disorders

Graeme Black

Centre for Genomic Medicine, Manchester Academic Health Science Centre, Univ. of Manchester, Central Manchester Univ. Hospitals NHS Foundation Trust St. Mary's Hospital, Manchester, UK

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

Institute of Medical Molecular Genetics, Zurich, Switzerland

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 themolecular 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

Lonneke Haer-Wigman

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 is becoming 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.

Literature:

Next-generation genetic testing for retinitis pigmentosa.

Kornelia Neveling, Rob W.J. Collin, Christian Gilissen, Ramon A.C. van Huet, Linda Visser, Michael P. Kwint, Sabine J. Gijsen, Marijke N. Zonneveld, Nienke Wieskamp, Joep de Ligt, Anna M. Siemiatkowska, Lies H. Hoefsloot, Michael F. Buckley, Ulrich Kellner, Kari E. Branham, Anneke I. den Hollander, Alexander Hoischen, Carel Hoyng, B. Jeroen Klevering, L. Ingeborgh van den Born, Joris A. Veltman, Frans P.M. Cremers and Hans Scheffer. *Hum Mutat. 2012 Jun;33(6):963-72. doi: 10.1002/humu.22045. Epub 2012 Mar 19. Erratum in: Hum Mutat. 2013 Aug;34(8):1181.*

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
Accepted manuscript online: 30 SEP 2013 09:16AM EST | DOI: 10.1002/humu.22450

Literature:
Next-generation genetic testing for retinitis pigmentosa.
Kornelia Neveling, Rob W.J. Collin, Christian Gilissen, Ramon A.C. van Huet, Linda Visser, Michael P. Kwint, Sabine J. Gijsen, Marijke N. Zonneveld, Nienke Wieskamp, Joep de Ligt, Anna M. Siemiatkowska, Lies H. Hoefsloot, Michael F. Buckley, Ulrich Kellner, Kari E. Branham, Anneke I. den Hollander, Alexander Hoischen, Carel Hoyng, B. Jeroen Klevering, L. Ingeborgh van den Born, Joris A. Veltman, Frans P.M. Cremers and Hans Scheffer. *Hum Mutat. 2012 Jun;33(6):963-72. doi: 10.1002/humu.22045. Epub 2012 Mar 19. Erratum in: Hum Mutat. 2013 Aug;34(8):1181.*

A post-hoc comparison of the utility of Sanger sequencing and exome sequencing for the diagnosis of heterogeneous diseases
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Genetic Counseling in Inherited Eye Disease

Ms Georgina Hall and Dr Marco Seri

Central Manchester and Manchester Children's Foundation Trust, Manchester, UK
Dept. of Medical Genetics, Sant'Orsola Malpighi, 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, 28th

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

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

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 21 genes known for ADRP, 34 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 19 genes so far identified, *GUCY2D*, *RPE65*, *CRX*, *AIPL1*, *CRB1*, *RPGRIP1*, *MERTK*, *RDH12*, *IMPDH1*, *TULP1*, *CEP290*, *LCA5*, *SPATA7*, *OTX2*, *IQCB1*, *PDE6G*, *KCNJ13*, *NMNAT1*, *RD3* & *DTHD1* 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 12 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

UCL Institute of Child Health, University College London, UK

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 that may be applicable to a broad range of retinal degenerations. 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. Stem cell therapies offer a complementary approach that may be more broadly. Pluripotent stem cell lines offer the potential to generate unlimited quantities of new retinal cells for transplantation. Remarkably, recent studies have shown that it is possible to generate new synthetic retina from cultures of embryonic stem cells. Ongoing 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 work has identified from the developing mouse retina an optimal donor cell population that following sub-retinal transplantation generates large numbers of integrated new rod photoreceptor cells in adult mice retinae. Successful integration of new cells was only achieved by transplanting immature precursor cells committed to a photoreceptor lineage. This same type of donor cell can be isolated from embryonic stem cell cultures. We have shown that photoreceptor precursors can be transplanted in a range of models of inherited retinal dystrophies, and restore rod-mediated vision in a genetic model of night-blindness. These findings indicate the feasibility of photoreceptor transplantation as a therapeutic strategy for restoring vision after retinal degeneration. Current and future challenges for the development of photoreceptor cell replacement therapies for retinal disease will be discussed.

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Photoreceptor precursors derived from three-dimensional embryonic stem cell cultures integrate and mature within adult degenerate retina.
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Genetics Basis of Age-Related Macular Degeneration (AMD)

Rando Allikmets

Dept. of Ophthalmology, Columbia University, USA

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 eight 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 proportion of the disease burden, making AMD one of the most well-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] 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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AAV Vectors for Retinal Gene Therapy

Alberto Auricchio

Telethon Institute of Genetics and Medicine, Napoli – Italy

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.

September, 29th

Architecture of Genetic Disease: Causes, Modifiers and the Concept of Genetic Load

Retinal Ciliopathies: Diverse Phenotyopes with Overlapping Genetic Structure

Nicholas Katsanis

Dept. of Cell Biology & Center For Human Disease Modeling
Duke University School of Medicine, Durham, USA

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.

Genetics of Glaucoma

Jane C Sowden

UCL Institute of Child Health, University College London, UK

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 leading to 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 several 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. For example, CDKN2BAS was identified as a risk factor contributing to CDR and TMOC1 for IOP, as well as several additional new genetic risk factors. 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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Overview of Developmental Eye Anomalies

Graeme Black

Centre for Genomic Medicine, Manchester Academic Health Science Centre, Univ. Of Manchester, Central Manchester Univ. Hospitals NHS Foundation Trust St. Mary's Hospital, Manchester, UK

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.

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

Sandro Banfi

Dipartimento di Biochimica, Biofisica e Patologia generale, Second University of Naples (SUN), Naples, and Telethon Institute of Genetics and Medicine (TIGEM), Pozzuoli, Italy.

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 are now determining the expression profiles of miRNAs in the human retina by Next Generation Sequencing procedures. 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 (4-5). 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 (6). 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

F.P.M. Cremers

PhD, Department of Human Genetics, Radboud University Medical Center, Nijmegen, The Netherlands.

Inherited retinal diseases display a high degree of clinical and genetic heterogeneity. Mutations in different genes can have different effects on the clinical phenotype, but the mutational spectrum in a given gene also can be substantial. Though autosomal recessive mutations are generally considered loss-of-function mutations, mutations with small effects, the so-called hypomorphic or mild mutations, are relatively common. 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.

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. submitted).

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Genetics of Mitochondrial Diseases & Retinopathies

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

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

Piero Barboni

Studio D'Azeglio, Bologna, Italy

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 2015

Valerio Carelli

Lab. Of Neurogenetics, University of Bologna, Italy

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 exploit 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 there are reasons to believe that 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 therapy with anti-oxidants will not be the ultimate problem-solving strategy, and other options should be adopted, in particular for the acute phase of LHON. Overall, there is a change in the paradigm, shifting from the untreatable condition to the availability of some treatments.

ABSTRACTS OF STUDENTS POSTERS

Identification of RCBTB1 as novel disease gene for autosomal recessive retinitis pigmentosa associated with systemic features

Giulia Ascari1*, Frauke Coppieters1*, Marcus Karlstetter2, Nina De Rocker1, Morgane Van der Eecken1, Miriam Bauwens1, Andy Willaert1, Annekatrien Boel1, Marieke De Bruyne1, Thalia Van Laethem1, Kris Vleminckx1, Françoise Meire5, Bart P. Leroy1,3,4, Thomas Langmann2, Elfride De Baere1.

*These authors contributed equally to this work

1Center for Medical Genetics Ghent, Ghent University, Ghent, Belgium;

2Department of Ophthalmology, University of Cologne, Cologne, Germany;

3Department of Ophthalmology, Ghent University Hospital, Ghent, Belgium;

4Division of Ophthalmology, The Children's Hospital of Philadelphia, Philadelphia, PA, USA;

5Department of Ophthalmology, Queen Fabiola Children's University Hospital, Brussels, Belgium.

Purpose: To identify and functionally study a novel disease gene mutated in a Turkish consanguineous family with autosomal recessive retinitis pigmentosa (arRP), associated with hypothyroidism, hypogonadism (amenorrhea), short stature, intellectual disability, and facial dysmorphism.

Methods: Genome-wide SNP arrays were used for homozygosity mapping in three affected and one healthy sibling of one consanguineous family. Two affected and one healthy individual underwent whole exome sequencing (WES) (HiSeq2000, Illumina, CLC bio). Segregation analysis of variants was done using Sanger sequencing. RCBTB1 expression was assessed using commercial human cDNAs. Immunostaining was carried out in mouse and human retina using a commercial anti-RCBTB1 antibody (abcam). Rcbtb1 in situ hybridization was performed in zebrafish. Morpholino and CRISPR/Cas9 experiments in zebrafish are ongoing.

Results: Homozygosity mapping revealed a single homozygous region on chromosome 13 (hg19: chr13: 41820714-53537171) shared by the three affected individuals of two branches of the family. WES identified a missense variant, c.973C>T p.(His325Tyr) (rs200826424, MAF 0.1%), in RCBTB1 (NM_018191.3). This variant was found to be homozygous in the three affected individuals and heterozygous in the healthy sibling and in the parents of the affected persons. Another family member with polydactily, but without retinal involvement, was heterozygous for c.973C>T even so no second RCBTB1 mutation was found, suggesting another condition in this family. Strong conservation of the His325 residue and different prediction tools suggest an effect of the missense variant on protein function. RCBTB1 expression was demonstrated in human retinal tissues. Subtle RCBTB1 immunoreactivity was shown in the inner retina. Preliminary in situ hybridization in zebrafish showed a staining restricted to the head with no apparent expression in other systems. Reinspection of the WES data is ongoing to exclude mutations in other genes located or not in the homozygous region that may underly the systemic features in this family. In addition sequencing of RCBTB1 in a larger cohort of arRP patients is ongoing.

Conclusions: A causal missense variant was identified in the RCBTB1 gene, encoding the regulator of chromosome condensation (RCC1) and BTB (POZ) domain containing protein 1. Interestingly, RCC1-like domains are also present in NEK8 and RPGR, which are known ciliary proteins implicated in
nephronophthisis and XLRP, respectively. Further functional characterization of RCBTB1 will provide more insights in its role in blinding disease.

A Case of Progressive Hearing and Visual Impairment: the Utility of Exome Sequencing

Emma Bedoukian

MS CGC, The Children's Hospital of Philadelphia

This case exemplifies how exome sequencing can be used to find a unifying diagnosis for a patient with multiple system involvement. This patient is a now 11 year old female. She was born following an unremarkable pregnancy to a healthy mother. She passed her newborn hearing screen, her metabolic screening, and she went home after two days. She presented to the otolaryngologist at 6 years old with complaints of hearing loss. She was fitted with hearing aids bilaterally which did help. About one year later she presented to ophthalmology for a baseline exam, which is routine when a patient is diagnosed with hearing loss at our institution. The ophthalmologist took a history and found that the patient did have blurred vision, he accounted for this by her near-sightedness and stated it was unrelated to her hearing loss. She did not have a retinal or optic nerve problem, she was prescribed new glasses. Also part of the routine work-up for a patient with hearing loss is a genetics evaluation. The work-up was completed and unremarkable: array normal and no mutations in common hearing loss genes (over 70 tested). Follow-up was recommended in genetics if symptoms changed or worsened. Two years later the patient's hearing had declined so much that cochlear implantation was required. She did not feel her glasses were helping anymore with her vision. She re-presented to ophthalmology. Her exam was remarkable for finger counting only in both eyes and bilateral optic atrophy which was not present at her prior visit. This worsening of hearing loss and vision prompted a follow-up genetics evaluation. Genetics reviewed all systems and in addition to worsening of hearing and vision noted degrading neurologic status. At this evaluation the Individualized Medical Genetics Center recommended exome sequencing, which was performed as a trio with both of the patient's parents. Exome sequencing revealed compound heterozygous mutations in SLC25A2. One of the identified mutations was novel, and one has been previously reported. This is consistent with Brown-Vialetto-Van-Laere syndrome 2, also known as Riboflavin transporter deficiency neuronopathy type 2. We counseled the family regarding the implications and medical associations with riboflavin transporter neuronopathy. Children with riboflavin transporter neuronopathy can have early onset sensorineural deafness, weak facial muscles, severe diffuse muscle weakness and wasting resulting in respiratory insufficiency, progressive optic atrophy, vision loss, progressive pontobulbar palsy, and loss of independent ambulation. Riboflavin transporter neuronopathy is due to an abnormal riboflavin transporter and cannot be cured, but can be treated with high-dose oral supplementation of riboflavin. Riboflavin is a vitamin that is important in the body's utilization of energy. When untreated, riboflavin transporter neuronopathy is often lethal. We believe this case exemplifies the utility of exome sequencing. It identified a disorder that is hereditary and treatable. The patient has two younger siblings so this becomes important not only for her, but her siblings and other family members as well. This is also an example of a rare disorder that should be always considered in our clinic patients with optic atrophy since it is treatable.

Genetics of Pseudoexfoliative Glaucoma: a Whole Exome Sequencing Approach

Julia Camilleri

University of Malta

Pseudoexfoliative glaucoma refers to the ocular manifestation of pseudoexfoliation syndrome, a systemic disorder characterised by accumulation and deposition of an abnormal fibrillar material. The condition is thought to result from abnormalities in extracellular matrix synthesis and metabolism, yet the pathogenesis is still unclear. Mutations in different genes have been implicated in association with the disease, and environmental factors may also play a role. Through whole exome sequencing of related cases of pseudoexfoliative glaucoma, important information on the genetic aetiology of the condition can be obtained.

The JR5558 Mouse Model of Spontaneous Neovascularization: Phenotype and Initial Genetic Analysis

R.H.Foxton

University College London, Institute of Ophthalmology, London, UK

The JR5558 mouse (see Nagai et al, IOVS 2014 55: 3709-3719) develops bilateral spontaneous choroidal neovascularization (sCNV), starting between approximately post-natal day 10-15 (P15), which increases in number and severity with age. Using fluorescein angiography, ocular coherence tomography (OCT), ERG, immunostaining, biochemistry and electron microscopy, we characterized the phenotype of the mouse, and discovered a pathology that involved multiple tissues, including disruption and dysfunction of vasculature, neurons, macrophages, glia and RPE.
A number of morphological methods confirmed the choroidal origin and subretinal position of the angiogenic vessels. At approximately P25, vessels were present in the outer retina with instances of anastomosis of some lesions with the retinal vasculature. The number of CNV lesions was significantly decreased by systemic blockade of the VEGF-A pathway. CNV size also was significantly modulated by reducing the number of lesion-associated macrophages. Later stages of sCNV are associated with edema, neuronal loss, and dysfunction.
Currently the genetics responsible for the mouse phenotype are unknown, but likely involve multiple genes, with autosomal recessive inheritance. We have carried out transcriptome analysis to identify variants, as well as backcrossing the mouse onto a C57Bl/6J background to hopefully identify causative mutations more rapidly.

Genome-Wide Homozygosity Scan Mapped Novel Protein Truncating Mutations in Consanguineous Pakistani Families Segregating Retinal Disorders

Muzammil Ahmad Khan 1,3, Sumitra Mohan 2, Katharina Gehmacher 2, Saadullah Khan 3, Zafar Nawaz 3, Ramzi Mohammad 3, Christian Windpassinger 2

1 Gomal Centre of Biochemistry and Biotechnology, Gomal University Dera Ismail Khan, Khyber-Pakhtoonkhwa 29050, Pakistan
2 Institute of Human Genetics, Graz Medical University, Graz 8010, Austria
3 Genomic Core Facility, Interim Translational Research Institute, Academic Health System, Hamad Medical Corporation, Doha, 3050, Qatar

Correspondence should be addressed to!
Christian Windpassinger, Associate Professor, Institute of Human Genetics, Graz Medical University, Graz, Austria
Email: christian.windpassinger@medunigraz.at
Phone: +(43)-3163804114
Fax: +(43)-3163809605

Keywords: Retinitis Pigmentosa, BBS syndrome, Consanguinity, SNP microarray, Homozygosity Mapping, BBS9 gene, LCA5 gene, Protein truncating mutation

Inherited retinal dystrophies are the leading cause of visual impairment among the people of developing countries. Retina is a photo-sensitive layer of cells that is responsible for visual perception. Degeneration of these photo-receptors is the ultimate cause of progressive vision loss either early in the life or in the later stages.

Here we report novel deletion mutations in two consanguineous Pakistani families, presenting autosomal recessive retinal disorders. The clinical interpretation revealed the segregation of Bardet Biedl syndrome (**BBS**) and Retinitis Pigmentosa (**RP**) in these families. The cardinal features of BBS patients presented retinitis pigmentosa, synpolydactyly, obesity, intellectual disability and renal abnormality, while the other family was suffering with progressive vision loss.

Genome wide SNP genotyping followed by sanger DNA sequencing found *BBS9/PTHB1* gene defect in patients with BBS syndrome, while the RP patients revealed a pathogenic variant in *LCA5* gene. The gene *BBS9* showed a single base deletion mutation [(c.299delC) (p. Ser100Leufs*24)] while the *LCA5* gene revealed 2 base pairs deletion [(c.1545_1546delAG) (p.Arg517Ilefs*3)]. These pathogenic variations shift the reading frame and producing premature stop codon which resulted in truncated proteins. The faulty *BBS9* encode 122 amino acid shortened peptide and completely loss its C-terminus PTHB1 domain, whereas the defective LCA5 after frame-shift produced 528 amino acid truncated protein and deleted C-terminus part.

Both the genes, *BBS9* and *LCA5*, encodes ciliary protein in which the defect of former has multi organ dysfunction, while later only affects the retinal cells.

Our medico-genetic data suggests the genetic heterogeneity of *BBS9* and *LCA5* gene in Pakistani population and may help in devising molecular diagnostic panel, which could be useful in genetic screening of sporadic cases with retinal dystrophies. Even then; a comprehensive study needs to be accomplished in order to elucidate the mystery of why defect in one ciliary gene has pleiotropic effects while the other affect only single tissue type.

Halting Progressive Neurodegeneration in Advanced Retinitis Pigmentosa

Susanne F. Koch, Yi-Ting Tsai, Jimmy K. Duong, Wen-Hsuan Wu, Chun-Wei Hsu, Wei-Pu Wu, Luis Bonet-Ponce, Chyuan-Sheng Lin, Stephen H. Tsang

Columbia University, USA

Retinitis pigmentosa (RP), a progressive neurodegenerative disease, is the most common cause of hereditary blindness in developed countries. There is currently no cure for RP (indeed, for any retinal degenerative disease). Of those therapies on the horizon, gene therapy may be the most promising. Retinal gene therapy has yet to achieve sustained functional rescue after disease onset – perhaps because transduction efficiency is insufficient ("too little") and/or the disease is too advanced ("too late") in humans. To test the latter hypothesis, we developed a novel mouse model for retinitis pigmentosa (RP) that allowed us to restore the mutant gene in all diseased photoreceptor cells, thereby ensuring sufficient transduction efficiency. We then treated mice at early, mid or late disease stages. At all three time points, degeneration was halted and function rescued for at least 1 year. In addition to being the first demonstration of gene therapy efficacy after onset of degeneration, our study is the first to demonstrate a broad therapeutic time window. The results suggest that RP patients are treatable, despite most being diagnosed after significant cell loss. To maximize clinical impact, our work suggests that gene therapy research must focus on improving transduction efficiency.

Coexisting Phenotypes and Non-Penetrance in a Family with *ABCA4* and *GPR143* Mutations

Winston Lee,[1] Yajing (Angela) Xie,[1] Kaspar Schurch,[1] Jana Zernant,[1] Stephen H. Tsang,[1,2] Janet R. Sparrow,[1,2] Rando Allikmets*[1,2]

[1]Department of Ophthalmology, Columbia University, New York, NY
[2]Department of Pathology & Cell Biology, Columbia University, New York, NY

*__Corresponding Author__: Rando Allikmets, Ph.D.
Dept of Ophthalmology
Eye Research Annex Rm 202
160 Ft Washington Ave
New York, NY 10032
Email: rla22@cumc.columbia.edu

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Keywords:

Abstract

Purpose: To describe the segregation and phenotypic expression of *ABCA4* and *GPR143* mutations in a single, two-generation family

Methods: A family consisting of five individuals (two parents and three children) were evaluated with quantitative 488nm and 787nm fundus autofluorescence imaging (AF) using a modified confocal scanning laser ophthalmoscope and electroretinogram testing (ERG). Complete sequencing of the *ABCA4* and *GPR143* gene was carried out in each individual.

Results: Two of the three children (daughters) presented with focal, optically empty lesions and perifoveal flecks while the maculae of the third child (son) and both parents were visibly unaffected. Full-field ERG testing revealed no generalized rod and cone dysfunction in the affected daughters. Quantitative autofluorescence levels were significantly elevated in the maculae of the affected daughters. Complete sequencing of *ABCA4* in the affected daughters revealed two disease-causing mutations, p.L541P; p.G1961E; mutational phase was confirmed in both parents. Furhter examination revealed a "mud-splattered" RPE pattern throughout the peripheral retina of the two affected daughters. Sequencing of GPR143 found a novel missense variant, c.A470G (p.Y157C). A re-examination of the father confirmed the absence of any retinal disease.

Conclusions: The two daughters exhibited a complex phenotype consisting of a focal variant of autosomal recessive Stargardt disease (STGD1) in the central macula and the classical carrier phenotype of peripheral RPE mosaicism in ocular albinism 1 (OA1) due to random X chromosome inactivation. Sequencing confirmed the segregation of two *ABCA4* mutations in the affected daughters as well as the presence of a novel *GPR143* mutation segregating from the father. The absence of disease expression in the father is uncertain, though may reflect an instance of familial non-penetrance or phenotypic rescue.

Genetic Counseling for Inherited Eye Disorders – Experience from a Multi-Cultural Society

Michal Macarov, Anat Blumenfeld, Dror Sharon, Claudia Yahalom

Department of Ophthalmology, Hadassah-Hebrew University Medical Center, Jerusalem, Israel

The Michaelson Institute for Rehabilitation of Vision offers services to children and adults with low vision, using a multidisciplinary approach. The staff is comprised of ophthalmologists, optometrists, two genetic counselors and a social worker. It is an integral part of the Department of Ophthalmology at Hadassah Medical Center and serves approximately 1400 patients annually, 65% of them are under the age of 18.

The in-house genetic counseling service is unique and does not exist in other departments at Hadassah Medical Center, apart from Oncology. The patients and their relatives benefit from a "one stop shop" and from a genetic counselor that specializes in ocular genetics and is familiar with the available genetic tests and on-going research.
The Michaelson institute and the molecular ophthalmology research and genetic testing labs are located in close proximity. This enables the researchers to meet the patients and keep them updated about advances in the research. When the genetic cause for a patient's disease is identified in the

research lab, the patient is referred for genetic counseling and the finding is validated in the clinical lab.
A unique challenge for our genetic counseling service stems from the multi-cultural nature of Israeli society: First, the Israeli population is made up of various ethnic groups. Founder mutations were identified in some ethnic groups, and high rates of consanguinity and intracommunity marriages lead to high incidence of autosomal-recessive ocular diseases. Second, varied levels of religious observance and practice exist in each of the different ethnic groups. Taken together, genetic information provided to counselees is often used for genetic tests and procedures that are uncommon in other populations. For example: premarital carrier tests and Pre-implantation Genetic Diagnosis (PGD) are performed for albinism among the ultra-orthodox Jews. We will present examples of genetic counseling and testing performed for prevalent inherited eye disorders in the Israeli population, and related genetic counseling issues.

Two Novel PRPF31 Mutations, Including a Deletion and a Duplication, in the Same Spanish Family Affected with Retinitis Pigmentosa

Martin Merida Maria Immaculada

Fundación Jiménez Díaz, Madrid, Spain

Introduction
Retinitis Pigmentosa (RP) is the most common form of the hereditary retinal dystrophies, with a prevalence of 1/4000. It is characterized by rod photoreceptors death that causes the reduction of visual field, pigment on fundus examination, and night blindness at the early stages, and a great clinical and genetic heterogeneity (1).
PRPF31 (19q13.42, human homolog of yeast pre-mRNA splicing factor 31) is one of the three pre-mRNA splicing factors that encode components of the spliceosomeU4/U6*U5 tri-snRNP and is one of the 26 disease causing genes of autosomal dominant RP with a prevalence of 1.7-6.7% depending on the populations (2). To date a total of 99 mutations in this gene have been described in RP (Human Genome Mutation Database), including splicing mutations, deletions, missense and nonsense mutations, insertions and indels. Also, genomic rearrangements have been described (3).
Objective
To search for genomic rearrangements in PRPF31 among previously studied and uncharacterized autosomal dominant Retinitis Piegmentosa (adRP) unrelated families.
Materials and methods
Affected individuals from all families underwent ophthalmic examination. Diagnosis of adRP was determined according to a dominant mode of inheritance in patients with night blindness, peripheral vision loss, ocular fundus alteration and reduced ERG scotopic response.
A total of 43 adRP Spanish families, previously studied by a NGS-based approach with a custom gene panel related to retinal dystrophies (Haloplex capture technology -Agilent Technologies Inc., Santa Clara, CA, USA-) (4), were selected to screen for deletions/duplications in RP genes using Multiplex ligation dependent probe amplification (MLPA P235, Retinitis Pigmentosa Kit, MRC-Holland). The MLPA was analyzed with the Coffalyser (MRC-Holland) software.
In the positive cases, and in order to define the breakpoint regions, a comparative genomic hybridation CGH array (SurePrint G3 CGH+SNP Posnatal 2X 400k -Agilent Technologies-) and a Long-PCR (Expand Long Range dNTPack –Roche-) was performed.

Results

After the screening among the 43 families, two novel mutations in the PRPF31 gene were identified in two different branches of the same family. One of the branches presented a heterozygous duplication encompassing the exons from 2 to 5 and the other one a heterozygous deletion including the exons 1 to 13.

The aCGH allowed us to delimit the duplication and deletion region and the Long-PCR showed us that the duplication region was contiguous and intragenic.

The deletion had 29748 bp and encompassed part of OSCAR gene, NDUFA3 gene, TFPT gene and part of PRPF31 gene, meanwhile the duplication had 5140 bp and encompassed part of PRPF31 gene.

Discussion and Conclusions

PRPF31 is one of the most important gene mutated in adRP (5), where genomic rearrangements are described because chromosome 19 is the most Alu-rich chromosome (6) which could explain the genomic rearrangements by non-allelic homologous recombination between two Alu regions as was explained previously (7). Haloplex NGS panel did not display the duplication or deletion in this family, for this reason it is necessary to use alternative techniques that allow to recognize gross deletions and insertions in this gene in negative NGS cases.

Disease mechanism is caused by haploinsufficiency (2) therefore we expect that the expression in both branches of the family tree, with duplication or deletion in PRPF31 gene, have a decrease level of this gene expression due to the fact that in both situation a premature stop codon can occur, but we need more studies and their correlation with the phenotype.

It is important diagnosis of RP at the molecular level because this allows a more precise prognosis of the future clinical evolution, it can be followed by genetic counseling and it is crucial for the inclusion in human gene-specific clinical trials.

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Identification of a Novel Mutation Confirms the Implication of IFT172 in Bardet-Biedl Syndrome (BBS20)

E. Schaefer1,2, **J. Muller**3,4, S. Scheidecker1, V. Geoffroy1, M. Prasad1, C. Redin4, I. Missotte5, D. Lacombe6, J.L. Mandel3,4,7, C. Stoetzel1, H. Dollfus1,[2]

1Laboratoire de Génétique Médicale, Institut de Génétique Médicale d'Alsace, INSERM U1112, Fédération de Médecine Translationnelle de Strasbourg (FMTS), Université de Strasbourg, Strasbourg, France.
2Service de Génétique Médicale, Hôpitaux Universitaires de Strasbourg, Strasbourg, France
3Laboratoire de Diagnostic Génétique, Hôpitaux Universitaires de Strasbourg, Strasbourg, France
4Département de Médecine Translationnelle et Neurogénétique, IGBMC, CNRS UMR 7104/INSERM U964/Université de Strasbourg, Illkirch, France
5 Service de Pédiatrie, Centre Hospitalier de Nouvelle-Calédonie, Hôpital de Magenta, Nouméa.
6 CHU de Bordeaux, Génétique Médicale, Université de Bordeaux, Laboratoire MRGM, Bordeaux, France
7Chaire de Génétique Humaine, Collège de France, Illkirch, France

Background

Bardet-Biedl Syndrome (BBS; MIM 209900) is a recessive and genetically heterogeneous syndromic retinopathy characterized by retinitis pigmentosa, postaxial polydactyly, obesity, hypogonadism, cognitive impairment and kidney dysfunction. So far, 20 BBS genes have been identified, with the last reported ones being found in one or very few families.

Methods and Results

Whole exome sequencing and homozygosity mapping was performed in a consanguineous family in which two affected children presented typical BBS features (retinitis pigmentosa, postaxial polydactyly, obesity, hypogonadism and cognitive impairment) without mutation identified in known BBS genes at the time of the study. We identified in the last reported BBS gene, namely *IFT172*, a homozygous splice-site mutation (NM_015662.2: c.4428+3A>G) homozygous in both the two patients in the last reported BBS gene, namely IFT172. Familial mutation segregation of the mutation was consistent with autosomal recessive inheritance. *IFT172* mutations have been initially reported in Jeune and Mainzer-Saldino syndromes (Halbritter et al., 2013). Recently, mutations have also been found in isolated retinitis pigmentosa and Bardet-Biedl-like ciliopathy (Bujakowska and al., 2014). This is the second report of *IFT172* mutations in BBS patients confirming *IFT172* as a BBS gene. Moreover, another IFT gene, *IFT127*, was already associated with Bardet-Biedl syndrome (Aldahmesh et al., 2014) confirming the implication of IFT genes in the pathogenesis of BBS.

Conclusions

In this report we validate *IFT172* as the 20th BBS gene (*BBS20*)

Elucidation of the hidden genetic variation underlying Sorsby fundus dystrophy in a large Belgian pedigree: N-terminal TIMP3 mutation 15 years later.

Sarah Naessens1, Delfien Syx1, Sarah De Jaeghere1, Bart P Leroy1, 2, 3, De Baere Elfride1, Coppieters Frauke1

1Center for Medical Genetics Ghent, Ghent University, Ghent, Belgium;
2Department of Ophthalmology, Ghent University Hospital, Ghent, Belgium;
3Division of Ophthalmology, TheChildren's Hospital of Philadelphia, Philadelphia, PA, USA

Sorsby fundus dystrophy (SFD) is an autosomal dominant retinal dystrophy in which patients lose central vision during the fourth or fifth decade of life. SFD is caused by mutations in TIMP3, the majority of which are missense mutations. TIMP3 is a member of a family of four secreted proteins, inhibiting the activity of matrix metalloproteinases. In 2000, Assink et al. (Br J Ophthalmol, 2000) examined a large Belgian family with typical SFD and investigated whether or not TIMP3 was involved. Although linkage was found with the TIMP3 locus 22q12.1-q13.2, mutation screening using single strand conformational polymorphism (SSCP), manual Sanger sequencing and cloning of the fifth exon of TIMP3 did not reveal any mutation in the coding exons, intron-exon boundaries, promotor region and 3'UTR of TIMP3. Here, it was our aim to elucidate the genetic cause of SFD in this family. Therefore, we performed microsatellite segregation analysis with four additional markers located closer to the gene. We confirmed linkage with TIMP3, and performed automated Sanger sequencing of the coding region of TIMP3, revealing a known mutation in exon 5, c.113C>G p.(Ser38Cys). This is the only known mutation located in the N-terminal domain of the TIMP3 protein. Segregation analysis in 63 family members showed co-segregation of this mutation with the disease in the family. TIMP3 comprises six disulfide bonds, formed by twelve cysteines. The p.(Ser38Cys) mutation results in an unpaired cysteine in the N-terminus. Arris et al. 2003 (Biochimica et Biophysica Acta, 2003) have proposed a mechanism whereby mutant proteins form abnormal disulfide-bonded dimers and aggregates that retard the turnover of the protein in Bruch's membrane. To investigate whether Nterminal mutation p.(Ser38Cys) results in abnormal disulfide-bonded dimers, Western blot analysis on patient-derived fibroblasts is currently ongoing.
In conclusion, we elucidated the hidden genetic variation underlying SFD in a large Belgian pedigree, previously missed by less sensitive screening methods. Our study confirms the genetic homogeneity of SFD. In order to provide further insight into the effect of the N-terminal TIMP3 mutation, functional characterization is ongoing.

A Novel Missense Mutation in a Malaysian Family with Autosomal Dominant Nystagmus and Presenile Cataracts Adds to the Existing Spectrum of PAX6 Mutations

Winnie Peitee Ong1, Rachel Gillespie2, Graeme Black2, Wee Teik Keng1

1Department of Genetics, Kuala Lumpur Hospital, Malaysia.

2Department of Genetic Medicine, Central Manchester University Hospitals NHS Foundation Trust, St Mary's Hospital, Manchester, UK.

Paired box gene 6 (PAX6), a transcriptional regulator located at chromosome 11p13, is crucial for normal ocular and brain development. Although aniridia is the best known associated phenotype, heterozygous PAX6 mutations also cause various anterior segment malformations, foveal hypoplasia, cataract, nystagmus and high refractive errors.

We present a three generational Malaysian chinese family with autosomal dominant (AD) nystagmus and presenile cataracts. The proband is a 7 year-old girl, the elder of 2 children of non-consanguineous parents. She had congenital nystagmus at birth and ophthalmological assessment showed myopia and astigmatism. Her other eye structures were normal, with no iris abnormalities nor foveal hypoplasia. She had normal cognitive function and no dysmorphism. Her karyotype and MRI brain were normal. Interestingly, her 3 year-old brother had similar congenital nystagmus, and their father had presenile cataract in his 20's and nystagmus on lateral gaze. The paternal grandmother also had nystagmus and developed bilateral cataracts in her early 50's. All had normal anterior chambers and normal fundi. In this family, many other paternal relatives have nystagmus, with or without cataracts. There is no family history of learning difficulties.

Screening of 115 genes associated with congenital cataract and other lens abnormalities via NGS identified a heterozygous c.317G>C; p.(Arg106Pro) mutation in exon 7 of PAX6 gene, and this was confirmed using Sanger sequencing. The affected nucleotide and amino residues are highly conserved and reside within the Paired domain of the PAX6 encoded protein. This unreported variant is predicted to be pathogenic. Segregation studies for other family members can hopefully be done in future.

This family demonstrated AD non-syndromic congenital nystagmus with intrafamilial variability in the age of onset of cataracts, and absent iris abnormalities. In many reported PAX6 families with cataracts, aniridia was a key associated feature. Tzoulaki et al (2005) in studying genotype-phenotype correlations from the Human PAX6 Mutation Database found that mutations which lead to a premature termination codon are generally associated with aniridia, while missense mutations tend to cause non-aniridia phenotypes, as seen in our family. In 2014, Thomas et al reported a multigenerational British family with AD nystagmus, presenile cataracts and normal irides that segregated a novel missense PAX6 mutation, but the affected individuals also had foveal hypoplasia. This feature was absent in our family although we would need to examine more affected family members to be more certain.

We wish to highlight that patients with AD nystagmus and cataracts, even in the presence of normal iris structure, should be considered for PAX6 screening. The finding of a novel mutation not only adds to the existing spectrum of PAX6 mutations but also alerts us to look hard for other PAX6-associated ophthalmological and neurological features in our family.

Whole Exome Sequencing Reveals ZNF408 as a New Gene Associated With Autosomal Recessive Retinitis Pigmentosa with Vitreal Alterations

Raquel Perez-Carro*, Almudena Avila-Fernandez*, Marta Corton, Maria Isabel Lopez-Molina,Laura Campello, Alex Garanto, Laura Fernadez-Sanchez, Lonneke Duijkers, Miguel Angel Lopez-Martinez, Rosa Riveiro-Alvarez, Luciana Rodrigues Jacy da Silva, Rocío Sanchez-Alcudia, Esther Martin-Garrido, Noelia Reyes, Francisco Garcia-Garcia, Joaquin Dopazo, Blanca Garcia-Sandoval, Rob W Collin, Nicolas Cuenca, Carmen Ayuso*.

Retinitis Pigmentosa (RP, MIM number 268000) is the most common form of inherited Retinal Dystrophies (RD) affecting 1 in 4,000 individuals 1. Symptoms include night blindness in the early phase of the disease, the development of tunnel vision, and slowly progressive decrease in central vision 2.

RP is inherited in all Mendelian forms: autosomal dominant, autosomal recessive or X-linked trait 3. Approximately 40% of patients with RP represent isolated cases. Non-Mendelian inheritance patterns such as digenic, mitochondrial or de novo mutations have been reported 4.

To date more than 80 genes have been described as causing non-syndromic autosomal recessive RP, and mutations in 55 genes have been identified as responsible of autosomal recessive RP (arRP). Since RP is heterogeneous, both clinically and genetically, is difficult to establish precise genotype-phenotype correlations.

Herein, homozygosity mapping and whole exome sequencing in a Spanish family with arRP led to the identification of a homozygous mutation (c.358_359delGT; p.Ala122Leufs*2) in the ZNF408 gene. Due to its expression in human retina and its implication in vasculature development previously described 5, the ZNF408 gene was selected as a new candidate gene causing arRP. A screening performed in 217 additional unrelated families revealed another homozygous mutation (c.1621C>T; p.Arg541Cys) in an isolated RP case.

Since this gene has been associated to Familial Exudative Vitreoretinopathy (FEVR [MIM number 133780]), a disorder affecting the growth and development of blood vessels in the retina 5, an intravenous fluorescein angiography (IVFA) was performed in homozygous and heterozygous carriers. The IVFA for the affected individuals showed classic RP findings and also vitreous alterations in some areas, blurring the visibility of the fundus. The asymptomatic heterozygous carriers were examined. No retinal vasculature abnormalities were observed in fundus or IVFA.

To shed light on the ZNF408 role in the retina and the pathogenesis of these mutations we have performed different functional studies. By immunohistochemical analysis in healthy human retina, we identified that ZNF408 is expressed in both cone and rod photoreceptors, in a specific type of amacrine and ganglion cells. ZNF408 encodes a transcription factor that harbors ten predicted C2H2-type fingers thought to be implicated in DNA binding. The immunochemistry experiments also revealed a cytoplasmic localization and a nuclear distribution in areas corresponding with the euchromatin fraction. Moreover, an assay in retinal blood vessels revealed the presence of the ZNF408 gene and therefore may be also involved in the retinal vascularization as previously described.

In order to discard a dominant-negative effect of the p.Arg541Cys missense variant, as was previously reported with the FEVR-associated p.His455Tyr variant 5, immunolocalization studies were performed and showed a partial mislocalization of the mutant protein retaining part of the wild-type protein in the cytoplasm resulting in the oligomerization of ZNF408 WT and mutant proteins.The p.Arg541Cys mutant ZNF408 also mainly localizes to the nucleus, but occasionally is also present in the cytoplasm of the transfected cells suggesting that the amount of mutant protein is not enough to produce any phenotypic alterations.

Interestingly, in this study novel homozygous mutations in the ZNF408 gene have been identified in two unrelated Spanish families as cause of RP. These findings support the hypothesis that different mutations, either in heterozygous or homozygous state, produce completely different phenotypes and suggest that ZNF408 may play additional roles apart from its implication in vasculature development. In this context, this is the first time the expression and cellular distribution of ZNF408 have been studied in the human retina.

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STUDENTS WHO IS WHO

SURNAME & NAME	AFFILIATION	NATIONALITY	CITY	E-MAIL
ASCARI GIULIA	Ghent University	ITALY	GHENT	giulia.ascari@ugent.be
BALIKOVA IRINA	Free University Brussels	BELGIUM	BRUXELLS	irina_balikova@yahoo.com
BEDOUKIAN EMMA	The Children's Hospital of Philadelphia	USA	PHILADELPHIA	ebedoukian@gmail.com
BODREAULT KATHERINE	Columbia University	USA	NEW YORK	Katherineboudreaull@hotmail.com
CAMILLERI JULIA ANNE	Univ. of Malta	MALTA	VICTORIA	jucamilleri@live.com
CARVALHO ANA LUISA	Centro Hosp. E Univ. de Coimbra	PORTUGAL	COIMBRA	aluisa.dcarvalho@gmail.com
DALLORTO LAURA	Clinica Oculistica Torino	ITALY	TORINO	laura.dallorto@gmail.com
D'ESPOSITO FABIANA	University of Naples Federico II	ITALY	NAPLES	fdesposito@alice.it
DIEMER TUE	Aalborg University Hospital	DENMARK	AALBORG	tuediemer@dadlnet.dk
EID OLA MOHAMED ABDELHAMID	National Research Centre	CAIRO	GIZA	olameid@hotmail.com
FOXTON RICHARD	University College London	UK	LONDON	richard.foxton@ucl.ac.uk
GESA ASTRID HAHN	Univ. Eye Hospital & Inst. For Ophthalmic Research	GERMANY	TUEBINGEN	gahahn.tue@googlemail.com
HARTMUT PETERS	ATG GenMed GmbH	GERMANY	BERLIN	hartmut.peters@yahoo.de
JIMENEZ ROLANDO BELEN	Hospital Cruz Roya	SPAIN	MADRID	belenjimnz@gmail.com
KARADEDOU CHRISTINA THEANO	DNA Health	GREECE	THESSALONIKI	karadedou.ct@gmail.com
KERAMATIPOUR MOHAMMAD	Tehran University of Medical Sciences	IRAN	TEHRAN	keramatipour@sina.tums.ac.ir
KESSEL LINE	Rigshospitalet – Glostrup. Dept. of Ophthalmology	DENMARK	GLOSTRUP	line.kessel@dadlnet.dk
KHAN MUZAMMIL AHMAD	Hamad Medical Corporation	QATAR	DOHA	muzammil.gandapur@hotmail.com
KOCH SUSANNE	Columbia University	USA	NEW YORK	susanne.f.koch@gmail.com
KUEHLEWEIN LAURA	Univ. Eye Hospital & Inst. For Ophthalmic Research	GERMANY	TUEBINGEN	Laura.Kuehlewein@med.uni-tuebingen.de
LEE WINSTON	Columbia University	USA	NEW YORK	wl2355@columbia.edu
MACAROV MICHAL	Hadassah-Hebrew University Medical Centre	ISRAEL	MODIIN	mmichal@hadassah.org.il

SURNAME & NAME	AFFILIATION	NATIONALITY	CITY	E-MAIL
MANITTO MARIA PIA	Ospedale San Raffaele	ITALY	MILANO	manitto.mariapia@hsr.it
MARCHESE CRISTIANA	AO Ordine Mauriziano	ITALY	TORINO	cmarchese@mauriziano.it
MARTIN MERIDA MARIA IMMACULADA	Fundación Jiménez Díaz	SPAIN	MADRID	inmamartinmerida@hotmail.com
MARYAM MOSTAFAVI	GENEVA CMU	SWITZERLAND	MONTREUX	maryamostafavi@gmail.com
MULLER JEAN	Hôpital Universitaire de Strasbourg	FRANCE	HOLTZHEIM	jeanmuller@unistra.fr
NAESSENS SARAH	Ugent	BELGIUM	GHENT	sarah.naessens@ugent.be
NOUPUU KALEV	Salzburg Medical Seminars in Ophthalmology	ESTONIA	TARTU	kalev.noupuu@gmail.com
ONG WINNIE PEITEE	Kuala Lumpur Hospital	MALAYSIA	SELANGOR	winnieong.pt@gmail.com
OOSTHUIZEN PIETER		SOUTH AFRICA	WOODHIL	pietmel@vodamail.co.za
PEREZ CARRO RACHEL	Fundación Jiménez Díaz Hospital	SPAIN	MADRID	raquel.perez@quironsalud.es
POLYAK MARGARITA	Petrovsky National Research Centre of Surgery	RUSSIA	MOSCOW	margaritapolyak@gmail.com
SCHÜRCH KASPAR	Columbia University	USA	NEW YORK	kws2118@cumc.columbia.edu
SPINETTA ROBERTA	Università di Torino	ITALY	TORINO	spinetta.roberta@gmail.com
VISMARA MARCO FLAVIO MICHELE	Univ. Tor Vergata	ITALY	ROME	marco@vismara.info

4th Course in Eye Genetics

FACULTY WHO IS WHO

Family Name	Name	Affiliation	Work e-mail
ALLIKMETS	**RANDO**	Dept. of Ophthalmology, Columbia University, USA	**rla22@cumc.columbia.edu**
AURICCHIO	**ALBERTO**	Telethon Institute of Genetics and Medicine, Napoli – Italy	**auricchio@tigem.it**
BANFI	**SANDRO**	Dept. di Biochimica, Biofisica e Patologia generale, Second University of Naples (SUN), Naples, and Telethon Institute of Genetics and Medicine (TIGEM), Pozzuoli, Italy	**banfi@tigem.it**
BARBONI	**PIERO**	Studio D'Azeglio, Bologna, Italy	**p.barboni@studiodazeglio.it**
BERGER	**WOLFGANG**	Institute of Medical Molecular Genetics – Zurich, Switzerland	**berger@medmolgen.uzh.ch**
BLACK	**GRAEME**	Centre for Genomic Medicine, Manchester Academic Health Science Centre, Univ. Of Manchester, Central Manchester Univ. Hospitals NHS Foundation Trust St. Mary's Hospital, Manchester, UK	**graeme.black@manchester.ac.uk**
CARELLI	**VALERIO**	Lab. Of Neurogenetics – Univ. of Bologna, Italy	**valerio.carelli@unibo.it**

Family Name	Name	Affiliation	Work e-mail
CIARDELLA	**ANTONIO**	Sant'Orsola Malpighi Hospital, Bologna, Italy	**antonio.ciardella@aosp.bo.it**
CREMERS	**FRANS**	PhD, Department of Human Genetics, Radboud University Medical Center, Nijmegen, The Netherlands.	**Frans.Cremers@radboudumc.nl**
GRAZIANO	**CLAUDIO**	Dept. of Medical Genetics, Sant'Orsola Malpighi Hospital, Bologna, Italy	**claudio.graziano@unibo.it**
HAER-WIGMAN	**LONNEKE**	Dept. of Human Genetics, Radboud University Medical Center, Nijmegen, The Netherlands	**Lonneke.Haer-Wigman@radboudumc.nl**
HALL	**GEORGINA**	Central Manchester and Manchester Children's Foundation Trust, Manchester, UK	**georgina.Hall@cmft.nhs.uk**
KATSANIS	**NICHOLAS**	Dept. of Cell Biology & Center for Human Disease Modeling, Duke University School of Medicine – Durham, USA	**nicholas.katsanis@duke.edu**
LEROY	**BART**	Dept. of Ophthalmology & CTR For Medical Genetics, Ghent University, Ghent – Belgium	**bart.leroy@ugent.be**
ROMEO	**GIOVANNI**	University of Bologna, European School of Genetic Medicine, Bologna, Italy	**egf.giovanni.romeo@gmail.com**
SERI	**MARCO**	Dept. of Medical Genetics, Sant'Orsola Malpighi Hospital, Bologna – Italy	**marco.seri@unibo.it**
SODI	**ANDREA**	Univ. of Florence, Italy	**asodi@tin.it**
SOWDEN	**JANE C.**	UCL Institute of Child Health, University College London, UK	**j.sowden@ich.ucl.ac.uk**