



European School of Genetic Medicine

3rd Course in Eye Genetics

Bertinoro, Italy, October 13-15 , 2013

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).



**3rd Course in
Eye Genetics**

Bertinoro, Italy, October 13 -15 , 2013

CONTENTS

• PROGRAMME	3
• ABSTRACTS OF LECTURES	6
• FACULTY WHO'S WHO	25
• STUDENTS' WHO'S WHO	27
• FURTHER READINGS	30

3RD COURSE IN EYE GENETICS

Bertinoro University Residential Centre Bertinoro, Italy, October 13-15 , 2013

Arrival day: Saturday, October 12th

October 13

9:00 - 9:10 **Welcome**

9:10 - 9:55 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

10:00 - 11:30 2 talks (40 min + 5 min discussion)

3. Genetics of glaucoma
Jane Sowden

4. IBD mapping in consanguineous and non-consanguineous families: finding retinal disease genes
Frans Cremers

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
Kornelia Neveling

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 & Neveling**)

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**)

October 14

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 corneal diseases
Graeme Black

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

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

4. Gene therapy for recessive and dominant eye diseases
Enrico Surace

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

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 & Neveling**)

October 15

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 AMD

Rando Allikmets

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. Modifier genes in retinal diseases

Frans Cremers

13:15-14:15 Lunch

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

15:45-16:15 break

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

6. Genetics of mitochondrial diseases and retinopathies

Bart Leroy

7. Mitochondrial optic neuropathies

Piero Barboni

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

Valerio Carelli

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

ABSTRACTS OF LECTURES

Sunday, October 13

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 25.000 and 30.000 genes. Of these, 246 genes (206 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

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. 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. It can be congenital, or with juvenile or adult onset. Genetic linkage studies in rare families showing Mendelian patterns of inheritance have identified 16 POAG genetic loci (GLC1A-Q) and four 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 seek 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 identified several genetic risk factors (SNPs located near new genes) that confer modest risk for glaucoma (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 in an attempt to further dissect the genetic components leading to disease susceptibility. Notably 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 all of 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.

[Reference: J. Wiggs 2012; IOVS 53, doi: 10.1167/iovs.12-9483e]

Identity-by-descent (IBD) mapping in consanguineous and non-consanguineous families facilitates the identification of novel retinal disease genes

F.P.M. Cremers

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

Inherited retinal diseases display a high degree of genetic heterogeneity, which renders the identification of the underlying disease genes very challenging. In the last two decades 41 genes have been identified in which mutations can give rise to autosomal recessive retinitis pigmentosa (arRP), the most frequent form of inherited blindness (den Hollander et al. 2010; <http://www.sph.uth.tmc.edu/RetNet/>). Together, these genes account for ~80% of the genetic causes of arRP, but most genes are mutated in only 1% of the cases. Genes underlying arRP have been identified using different strategies. About one-third of the genes has been identified based on their specialized function in the retina. Large cohorts of arRP patients were analysed systematically for mutations in these genes that play crucial roles in the phototransduction cascade or visual cycle. Another one-third of arRP genes was identified upon mutation analysis of human orthologs of genes that are mutated in animal models with inherited retinal diseases. Finally, one-third of arRP genes was identified using positional cloning strategies which often relied on homozygosity mapping in consanguineous families with arRP patients.

In the Dutch population, ~1/3 of patients with inherited retinal diseases carries homozygous mutations. This can be due to the fact that their parents both carry the same frequent mutation or that the parents share a common ancestor. The likelihood that they share a common ancestor is larger when the parents' ancestors originate from the same geographical region. It was hypothesized that the Dutch population contains subpopulations that until two generations ago, were relatively isolated. If new mutations arise in an isolated population, patients with autosomal recessive disorders in subsequent generations in most cases will carry a homozygous mutation. We reasoned that it may be possible to also use these 'nonconsanguineous' families for homozygosity or identity-by-descent (IBD) mapping. The advantage of these families could be that, due to the fact that parents share a common ancestor that lived between 5 and 15 generations ago, the IBD region in a patient will be relatively small due to the large number of genetic recombinations that occurred. As a rule of thumb, the size of a homozygous region is 100 cM (~100 Mb) divided by the number of generations between the patient and the common ancestor of the parents of the patient (Woods et al. 2006). To test this hypothesis, we performed genome-wide homozygosity mapping using high-density single nucleotide polymorphism (SNP) arrays in ~400 patients with arRP, Leber congenital amaurosis (LCA), cone- or cone-rod dystrophy (CD, CRD), or achromatopsia (ACHM). Patients were ascertained predominantly in the Netherlands, Canada, and Germany. In parallel, we and others performed IBD mapping in consanguineous families from Indonesia, Israel, and Pakistan. These studies revealed many patients from nonconsanguineous families with conspicuous homozygous regions. By comparing IBD mapping data with previously reported chromosomal loci for inherited retinal diseases, and by employing bioinformatic tools, we identified the elusive LCA gene *LCA5* (den Hollander et al. 2007) and the arRP gene *EYS* (Collin et al. 2008) located at the RP25 locus. The *LCA5* gene encodes Lebercilin, a critical component of the connecting cilium of

photoreceptor cells. The *EYS* gene is the human ortholog of *Drosophila eyes shut/spacemaker*, which in the fruitfly encodes a protein that is essential for photoreceptor morphology. This protein occupies the interrhabdomeral space of ommatidia, and in this way functionally separates the rhabdomers yielding a high visual spatial resolution. The human *EYS* gene measures 2,000 kb and thereby is the largest gene to be expressed specifically in the retina. Mutation screening revealed its involvement in ~5% of the arRP patients (Littink et al. 2010).

Using the same approach, in some studies in combination with next generation sequencing, we were able to identify one novel achromatopsia/cone dystrophy gene, *PDE6C* (Thiadens et al. 2009), five novel arRP genes, *C2orf71* (Collin et al. 2010), *IMPG2* (Bandah-Rozenfeld et al. 2010), *CLRN1* (Khan et al. 2011), *BBS1* (Estrada-Cuzcano et al. 2012a), and *C8orf37* (Estrada-Cuzcano et al. 2012b), as well as a novel arCRD gene (*RAB28*; Roosing et al. 2013).

In conclusion, homozygosity mapping in both consanguineous and nonconsanguineous families has proven to be effective in identifying novel retinal disease genes. In combination with next generation sequencing, this approach will allow us to rapidly identify the causal genes in other families with retinal dystrophies.

References:

- Bandah-Rozenfeld, D. et al. Mutations in *IMPG2*, encoding interphotoreceptor matrix proteoglycan 2, cause autosomal-recessive retinitis pigmentosa. (2010) *Am. J. Hum. Genet.* **87**, 199-208.
- Collin, R. W. J. et al. Identification of a 2 Mb human ortholog of *Drosophila eyes shut/spacemaker* that is mutated in patients with retinitis pigmentosa. (2008) *Am. J. Hum. Genet.* **83**, 594-603.
- Collin, R. W. J. et al. Mutations in *C2ORF71* cause autosomal-recessive retinitis pigmentosa. (2010) *Am. J. Hum. Genet.* **86**, 783-788.
- den Hollander, A. I., Black, A., Bennett, J. & Cremers, F. P. M. Lighting a candle in the dark: advances in genetics and gene therapy of recessive retinal dystrophies. (2010) *J. Clin. Invest.*, in press.
- den Hollander, A. I. et al Mutations in *LCA5*, encoding the ciliary protein lebercilin, cause Leber congenital amaurosis. (2007) *Nat. Genet.* **39**, 889-895.
- Estrada-Cuzcano, A. et al. (2012a) *BBS1* mutations underlie a wide spectrum of phenotypes ranging from nonsyndromic retinitis pigmentosa to Bardet-Biedl syndrome. *Arch. Ophthalmol.* **130**, 1425-1532.
- Estrada-Cuzcano, A. et al. Mutations in *C8orf37*, encoding a ciliary protein, are associated with autosomal-recessive retinal dystrophies with early macular involvement (2012b). *Am. J. Hum. Genet.* **90**, 102-109.
- Khan, M. I. et al. *CLRN1* mutations cause nonsyndromic retinitis pigmentosa. (2011) *Ophthalmology*, **118**, 1444-1448.
- Littink, K. W. et al. Mutations in the *EYS* gene account for ~5% of autosomal recessive retinitis pigmentosa and cause a fairly homogeneous phenotype. (2010) *Ophthalmology*, **51**, 3646-3652.
- Roosing, S. et al. Mutations in *RAB28*, encoding a farnesylated small GTPase, are associated with autosomal-recessive cone-rod dystrophy (2013). *Am. J. Hum. Genet.* **93**, 110-117. *Equal contributions.
- Thiadens, A. A. H. J. et al Homozygosity mapping reveals *PDE6C* mutations in patients with early-onset cone photoreceptor disorders. (2009) *Am. J. Hum. Genet.* **85**, 240-247.
- Woods, C. G. et al. Quantification of homozygosity in consanguineous individuals with autosomal recessive disease. (2006) *Am. J. Hum. Genet.* **78**, 889-896.

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

Wolfgang Berger

Institute of Medical Molecular Genetics, University of Zurich, 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 (Figure 1). The disease classification also considers whether the ocular phenotype is associated with pathologies of other tissues (syndromic forms) or only affects the retina, retinal pigment epithelium and the vitreous body (non-syndromic forms). In addition, the mode of inheritance is also used as one characteristic feature of the different disease phenotypes in order to categorize them.

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

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

Reference:

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

Introduction to next-generation sequencing for eye diseases

Kornelia Neveling, PhD

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

For many years Sanger sequencing has been the golden standard and workhorse in DNA diagnostic laboratories. However, implementing gene tests for all known monogenic diseases is impossible in single laboratories. Moreover, the cause of many genetic diseases is still unknown. Genetically heterogeneous disorders such as retinitis pigmentosa (RP) 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, whereas the extreme heterogeneity of RP does not allow Sanger sequencing of all known RP genes. Therefore, many patient samples currently remain genetically undiagnosed. Next generation sequencing (NGS) now opens the way to high throughput analysis of either targeted genomic regions or even the whole exome or genome and allow us for the first time to simultaneously sequence all known genes that are involved in RP.

Recently, targeted NGS was developed for all known inherited retinal disease genes, tested using DNAs of 12 retinal disease patients with different compound heterozygous variants and applied to 100 patients with autosomal recessive or isolated RP. In the cohort of 12 samples with known mutations, we were able to robustly identify 21/24 retinal disease-associated variants. In the cohort of 100 patients, 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 100 tested RP patients were derived from a larger cohort that was prescreened for mutations in selected retinal dystrophy genes, we estimated that this approach would find disease-causing mutations in ~50% of patients with isolated or autosomal recessive RP. Although this approach was very successful in the identification of disease-causing variants in a previously pre-screened cohort of retinal dystrophy patients, several disadvantages were linked to the method applied. We therefore now developed a generic workflow for diagnostic exome sequencing, with a focus on heterogeneous disorders such as hereditary blindness, but also hereditary deafness, mitochondrial diseases, movement disorders and cancer. In a first pilot experiment, we observed that the diagnostic yield of exome sequencing was as high as 52% for blindness, perfectly fitting to what we had calculated before.

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. Siemiakowska, 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

Genetic Counselling in Inherited Eye Disease

Georgina Hall and 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

Monday, October 14

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 heterogeneous 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 18 genes known for ADRP, 28 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*, *IQCBI*, *PDE6G*, *KCNJ13*, *NMNAT1* & *RD3* 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 applicable to replace lost or non functional cells. 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. Our work has identified from the developing mouse retina an optimal donor cell population that following sub-retinal transplantation generates large numbers of correctly integrated new rod photoreceptor cells in adult mice retinæ. 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 model of stationary 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.

Photoreceptor precursors derived from three-dimensional embryonic stem cell cultures integrate and mature within adult degenerate retina.

Gonzalez-Cordero A, West EL, Pearson RA, Duran Y, Carvalho LS, Chu CJ, Naeem A, Blackford SJ, Georgiadis A, Lakowski J, Hubank M, Smith AJ, Bainbridge JW, Sowden JC, Ali RR.

Nat Biotechnol. 2013 Aug;31(8):741-7.

Restoration of vision after transplantation of photoreceptors.

Pearson RA, Barber AC, Rizzi M, Hippert C, Xue T, West EL, Duran Y, Smith AJ, Chuang JZ, Azam SA, Luhmann UF, Benucci A, Sung CH, Bainbridge JW, Carandini M, Yau KW, Sowden JC, Ali RR.

Nature. 2012 May 3;485(7396):99-103.

Cone and rod photoreceptor transplantation in models of the childhood retinopathy Leber congenital amaurosis using flow-sorted Crx-positive donor cells.

Inherited Corneal Disorders

Graeme Black

Genetic 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 a transparent tissue that is, with the lens, the main refractive structure of the eye. It comprises an epithelium, a collagenous stroma and a non-dividing endothelial monolayer.

This lecture will give an overview of a range of inherited corneal abnormalities including:

Corneal dystrophies – a range of bilateral, progressive, non-inflammatory disorders. These may be classified by site (epithelial, stromal and endothelial) or by molecular defect.

In the first part of this talk the discovery of :

- i) keratin mutations underlying epithelial dystrophies
- ii) BIGH3, which underlies a range of epithelial and stromal disorders has been a key development
- iii) COL8A2 underlying endothelial dystrophies

Will be discussed.

Corneal clouding during childhood is another important group of disorders which may be caused by congenital glaucoma, early-onset dystrophic processes (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 will be discussed.

Gene therapy for recessive and dominant eye diseases

Enrico Surace

Telethon Institute of Genetics and Medicine, Naples – Italy

In the last decade a great effort has been put in both disease causative gene identification and to the design therapeutic approaches to treat inherited retinal degenerations.

Owed to the knowledge of retinal properties as a neuronal network, the relatively good risk/benefit ratio of therapeutic intervention, and the approachability of the retina, different therapeutic strategies have been attempted. However, one major hurdle to therapeutic intervention is genetic heterogeneity of retinal degenerations.

Among therapeutic paradigms, "gene therapy" due to its causative therapeutic design, in principle, holds great promise.

Depending on the genetic bases of inherited retinal degenerations different gene based approaches have been proposed and some are being tested in clinical trials.

The lecture will propose different "nucleic acid-based" therapeutic approaches employed and proof-of-concept tested for both recessive and dominant mode of inheritance of retinal degeneration as long as a recent example of a clinical trial study in which "gene therapy" has been successfully *translated* to human patients.

Tuesday, October 15

Genetic 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 disorders¹.

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 disease²⁻⁵. 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 CFH⁹. 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 locus¹⁰ and additional genetic and environmental factors. Another possibility is that the complement pathway and 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 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.

1. Dean, M. Approaches to identify genes for complex human diseases: lessons from Mendelian disorders. *Hum Mutat* **22**, 261-74 (2003).
2. Hageman, G.S. et al. A common haplotype in the complement regulatory gene factor H (*HF1/CFH*) predisposes individuals to age-related macular degeneration. *Proc Natl Acad Sci U S A* **102**, 7227-32 (2005).
3. Haines, J.L. et al. Complement factor H variant increases the risk of age-related macular degeneration. *Science* **308**, 419-21 (2005).

4. Klein, R.J. et al. Complement factor H polymorphism in age-related macular degeneration. *Science* **308**, 385-9 (2005).
5. Edwards, A.O. et al. Complement factor H polymorphism and age-related macular degeneration. *Science* **308**, 421-4 (2005).
6. Rivera, A. et al. Hypothetical LOC387715 is a second major susceptibility gene for age-related macular degeneration, contributing independently of complement factor H to disease risk. *Hum Mol Genet* **14**, 3227-36 (2005).
7. Jakobsdottir, J. et al. Susceptibility genes for age-related maculopathy on chromosome 10q26. *Am J Hum Genet* **77**, 389-407 (2005).
8. Gold, B. et al. Variation in factor B (BF) and complement component 2 (C2) genes is associated with age-related macular degeneration. *Nat Genet* **38**, 458-62 (2006).
9. Hageman, G. et al. Extended Haplotypes in the Complement Factor H (CFH) and CFH-related (CFHR) Family of Genes that Protect against Age-related Macular Degeneration: Identification, Ethnic Distribution and Evolutionary Implications. *Ann Med* **38**, 592-604 (2006).
10. Allikmets, R. and Dean, M. Bringing age-related macular degeneration into focus. *Nat Genet* **40**, 820-21 (2008).
11. Gehrs, K.M., et al. Complement, age-related macular degeneration and a vision of the future. *Arch Ophthalmol* **128**, 349-358 (2010)

Overview of developmental eye anomalies

Graeme Black

Genetic 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 Microphthalmia / Anophthalmia, including Coloboma or optic fissure closure defects, both Isolated and syndromic. Nanophthalmia
 Congenital Cataract, both Isolated and Syndromic.
 Anterior Segment Dysgenesis including Peters anomaly, Rieger anomaly and 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 principles behind gene mutations from visible chromosome abnormalities through to single gene deletions will be explored including the concepts of reduced expressivity, variable penetrance and genetic heterogeneity.

The role of non-coding RNAs in eye development, function and diseases

Sandro Banfi

Telethon Institute of Genetics and Medicine (TIGEM), and Dipartimento di Biochimica, Biofisica e Patologia generale, Second University of Naples (SUN), Naples, 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 the eye transcriptome is still largely unknown. To begin understanding their eye-related function in mammals, we looked for miRNAs significantly expressed in the mouse eye by means of high-resolution expression analysis. We analyzed the spatiotemporal localization of miRNAs in the murine embryonic and postnatal eye by RNA *in situ* hybridization (ISH) using LNA-modified oligonucleotide probes. Overall, we determined the expression of over 220 miRNAs and generated a high-resolution expression atlas of miRNAs in the developing and adult wild-type mouse eye. We found that 122 miRNAs displayed restricted expression domains in the eye at different developmental stages, with the majority of them expressed in one or more cell layers of the neural retina (3). We then selected, among the miRNAs showing the most interesting expression domains in the developing eye, miR-204, for *in vivo* functional analysis using the medaka fish (*Oryzias latipes*) model system. We demonstrated that miR-204 regulates multiple aspects of eye development in medaka. Morpholino-mediated ablation of miR-204 expression resulted in a striking eye phenotype that was characterized by microphthalmia, abnormal lens formation, and altered dorso-ventral patterning of the retina that is associated with optic-fissure coloboma. Using a variety of *in-vivo* and *in-vitro* approaches, we have identified several key targets for miR-204 function in the eye, among which the transcription factor *Meis2* and the *Ankrd13a* gene. We showed that together with altered regulation of the Pax6 pathway, the abnormally elevated levels of *Meis2* resulting from miR-204 inactivation are largely responsible for the observed phenotype. These data provide the first example of how a specific microRNA can regulate multiple events in eye formation (4-5).

REFERENCES

- (1) He L, Hannon GJ (2004) MicroRNAs: small RNAs with a big role in gene regulation. *Nat Rev Genet* 5:522-531.
- (2) Meola N, Gennarino VA, **Banfi S**. microRNAs and genetic diseases. *PathoGenetics* 2009, 2:7 (4 November 2009).
- (3) 76. Karali M, Peluso I, Gennarino VA, Bilio M, Verde R, Lago G, Dollé P and Banfi S. miRNeye: a microRNA expression atlas of the mouse eye. *BMC Genomics*. 2010 Dec 20;11:715.
- (4) Conte I, Carrella S, Avellino R, Karali M, Marco-Ferreres R, Bovolenta P, **Banfi S**. miR-204 is required for lens and retinal development via *Meis2* targeting. *Proc Natl Acad Sci U S A*. 2010 Aug 31;107(35):15491-6. Epub 2010 Aug 16.
- (5) Avellino R, Carrella S, Pirozzi M, Risolino M, Franco P, Stoppelli P, Verde P, **Banfi S***, Conte I*. MiR-204 targeting of *Ankrd13A* controls both mesenchymal neural crest and lens cell migration. *PLoS One*, 2013 Apr 19;8(4):e61099.

Modifier genes and digenic inheritance in retinal diseases

F.P.M. Cremers

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

Inherited retinal diseases display an enormous degree of clinical and genetic heterogeneity. Mutations in different genes can have different effects on the clinical phenotype, but the mutational spectrum within a given gene also can be large. 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 nongenetic 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 needed to elicit a clinical phenotype. In this lecture, five examples will illustrate allelic and non-allelic modifiers, as well as digenic inheritance.

PRPF31 and allelic modifier.

Mutations in the pre-mRNA splicing gene *PRPF31* were found in families with autosomal dominant retinitis pigmentosa (Vithana et al. 2001). However, many mutation-carriers were found to be asymptomatic. Detailed haplotype analysis and mRNA quantification studies 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 nonpenetrance.

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 two families with two BBS siblings and homozygous p.M390R mutations, Beales et al. (2003) found that the unaffected mothers were heterozygous for this variant and that the unaffected fathers were homozygous for this variant. In a subsequent study (Badano et al. 2006), a genetic modifier in *MGC1203 (BBSIP1)* was identified in both affected siblings in one of these families but not in the unaffected father. This variant, c.430C>T, had a moderate effect on the splicing of intron 2. In a comprehensive mutation analysis study, homozygous p.M390R variants were identified in 11 of 2,256 probands with RP and in one of 1,724 ethnically matched controls (A. Estrada-Cuzcano et al. submitted). 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.

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 carries the same *CEP290* variants, but shows a more severe phenotype, Leber congenital amaurosis. A second

cousin of these three patients shows 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. On the contrary, *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.

PDZD7 is a modifier of retinal disease and a contributor to digenic Usher syndrome

PDZD7 is homologous to Harmonin and Whirlin, which are mutated in patients with Usher syndrome (USH) types 1C and -2D, respectively. *PDZD7* was not found to be mutated in patients without mutations in one of the 9 known USH genes. On the contrary, in a family with two affected sisters and a homozygous *USH2A* stopmutation, a *de novo* stopmutation was found in *PDZD7* in the more severely affected sibling (Ebermann et al. 2010). A heterozygous *PDZD7* stopmutation was found in a USH patient with a heterozygous *GPR98* (*USH2C*) mutation, suggesting digenic inheritance. None of the *PDZD7* variants were found in more than 200 control individuals, but another *PDZD7* variant, c.2107delA, was found in 4/405 healthy controls. Morpholino-induced knock-down of one of two zebrafish homologues of *PDZD7*, *Pdzd7a*, resulted in reduced localization of *Gpr98* at the connecting cilium. Combined morpholino-induced knock-down of *Gpr98* and *Pdzd7a* resulted in a Usher-like circling and retinal degeneration, suggesting the functional interaction of the respective gene products.

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 retinal disease. In three families, Kajiwarra et al. (1994) identified non-allelic heterozygous variants in *PRPH2* (p.Leu185Pro) and *ROM1* (protein-truncating mutations). Biochemical studies revealed the disease mechanism underlying this digenic form of RP (Goldberg et al. 1995; Loewen et al. 2001). p.Leu185Pro-mutant *PRPH2* is unable to form *PRPH2* homotetramers, which, together with *ROM-1*, are needed for higher-order disulfide-linked oligomer formation. The levels of these oligomers is critical for stable photoreceptor disc formation.

References

- Badano, J.L., Leitch, C.C., Ansley, S.J., May-Simera, H., Lawson, S., Lewis, R.A., Beales, P.L., Dietz, H.C., Fisher, S., Katsanis, N. Dissection of epistasis in oligogenic Bardet-Biedl syndrome. (2006) *Nature* **439**, 326-330.
- Beales, P.L., Badano, J.L., Ross, A.J., Ansley, S.J., Hoskins, B.E., Kirsten, B., Mein, C.A., Froguel, P., Scambler, P.J., Lewis, R.A. et al. Genetic interaction of *BBS1* mutations with alleles at other *BBS* loci can result in non-Mendelian Bardet-Biedl syndrome. (2003) *Am. J. Hum. Genet.* **72**, 1187-1199.
- Ebermann, I., Phillips, J.B., Liebau, M.C., Koenekoop, R.K., Schermer, B., Lopez, I., Schafer, E., Roux, A.F., Dafinger, C., Bernd, A. et al. (2010). *PDZD7* is a modifier of retinal disease and a contributor to digenic Usher syndrome. (2010) *J. Clin. Invest.* **120**, 1812-1823.
- Goldberg, A.F., Moritz, O.L., Molday, R.S. Heterologous expression of photoreceptor peripherin/rds and Rom-1 in COS-1 cells: assembly, interactions, and localization of multisubunit complexes. (1995) *Biochemistry* **34**, 14213-14219.
- Kajiwarra, K., Berson, E.L., Dryja, T.P. Digenic retinitis pigmentosa due to mutations at the unlinked peripherin/*RDS* and *ROM1* loci. (1994) *Science* **264**, 1604-1608.
- Littink, K.W., Pott, J.W., Collin, R.W.J., Kroes, H.Y., Verheij, J.B., Blokland, E.A.W., de Castro Miro, M., Hoyng, C.B., Klaver, C.C.W., Koenekoop, R.K. et al. A novel nonsense mutation in CEP290 induces exon skipping and leads to a relatively mild retinal phenotype. (2010) *Invest. Ophthalmol. Vis. Sci.* **51**, 3646-3652.

- Loewen, C.J., Moritz, O.L., Molday, R.S. Molecular characterization of peripherin-2 and rom-1 mutants responsible for digenic retinitis pigmentosa. (2001) *J. Biol. Chem.* **276**, 22388-22396.
- Vithana, E.N., Abu-Safieh, L., Allen, M.J., Carey, A., Papaioannou, M., Chakarova, C., Al-Maghtheh, M., Ebenezer, N.D., Willis, C., Moore, A.T. et al. A human homolog of yeast pre-mRNA splicing gene, PRP31, underlies autosomal dominant retinitis pigmentosa on chromosome 19q13.4 (RP11). (2001) *Mol. Cell* **8**, 375-381.
- Vithana, E.N., Abu-Safieh, L., Pelosini, L., Winchester, E., Hornan, D., Bird, A.C., Hunt, D.M., Bustin, S.A., Bhattacharya, S.S. Expression of PRPF31 mRNA in patients with autosomal dominant retinitis pigmentosa: a molecular clue for incomplete penetrance? (2003) *Invest. Ophthalmol. Vis. Sci.* **44**, 4204-4209.

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 encode 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.

References:

RetNet on <http://www.sph.uth.tmc.edu/RetNet/>

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

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.

3rd Course in Eye Genetics

FACULTY Who is Who

Family Name	Name	Affiliation	Work e-mail
ALLIKMETS	RANDO	DEPT. OF OPHTHALMOLOGY, COLUMBIA UNIVERSITY - USA	rla22@columbia.edu
BANFI	SANDRO	TELETHON INSTITUTE OF GENETICS AND MEDICINE, NAPLES AND DIP. DI BIOCHIMICA, BIOFISICA E PATOLOGIA GENERALE, SECOND UNIVERSITY OF NAPLES (SUN), NAPLES – ITALY	banfi@tigem.it
BARBONI	PIERO	STUDIO D' AZEGLIO, BOLOGNA – ITALY	p.barboni@studiodazegio.it
BERGER	WOLFGANG	INSTITUTE OF MEDICAL GENETICS - UNIVERSITY OF ZURICH - SWISS	berger@medgen.uzh.ch
BLACK	GRAEME	GENETIC MEDICINE, MANCHESTER ACADEMIC HEALTH SCIENCE CENTRE, UNIV. OF MANCHESTER, CENTRAL MANCHESTER UNIV. HOSPITALS NHS FOUNDATION TRUST ST. MARY'S HOSPITAL.	graeme.black@manchester.ac.uk
CARELLI	VALERIO	LAB. OF NEUROGENETICS – UNIV. OF BOLOGNA, ITALY	valerio.carelli@unibo.it
CIARDELLA	ANTONIO	SANT'ORSOLA MALPIGHI HOSPITAL , BOLOGNA - ITALY	antonio.ciardella@aosp.bo.it

CREMERS	FRANS	DEPT. OF HUMAN GENETICS, Radboud University Medical Center - The Netherlands	f.cremers@antrg.umcn.nl
GRAZIANO	CLAUDIO	DEPT. OF MEDICAL GENETICS, SANT'ORSOLA MALPIGHI Hospital, Bologna - Italy	claudio.graziano@unibo.it
HALL	GEORGINA	CENTRAL MANCHESTER & MANCHESTER CHILDREN'S FOUNDATION TRUST	georgina.hall@cmft.nhs.uk
NICHOLAS	KATSANIS	INSTITUTE OF GENETIC MEDICINE, Johns Hopkins Hospital, Baltimore - USA	nicholas.katsanis@duke.edu
LEROY	BART	DEPT. OF OPHTHALMOLOGY & CTR FOR MEDICAL GENETICS, Ghent University, Ghent - Belgium	bart.leroy@ugent.be
NEVELING	KORNELIA	DEPT. OF HUMAN GENETICS, Radboud University Nijmegen Medical Centre	k.neveling@antrg.umcn.nl
SERI	MARCO	DEPT. OF MEDICAL GENETICS, SANT'ORSOLA MALPIGHI Hospital	marco.seri@unibo.it
SURACE	ENRICO	TELETHON INSTITUTE OF GENETICS AND MEDICINE, Naples - Italy	surace@tigem.it
SODI	ADRIANO	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

Who is Who: Participants

Family Name	Name	Affiliation	Nationality	City	E-mail
ALLMOALLEM	BASAMAT	CENTER FOR MEDICAL GENETICS	BELGIUM	GHENT	basamat.almoallem@ugent.be
ALOVISI	CAMILLA	UNIVERSITY EYE CLINIC	ITALY	TURIN	camillaalovisi@gmail.com
AMIT	TIWARI	INSTITUTE OF MEDICAL MOLECULAR GENETICS	SWITZERLAND	SCHWERZENBACH	tiwari@medmolgen.uzh.ch
BACCI	GIACOMO MARIA	AOU A. MEYER CHILDREN'S HOSPITAL	ITALY	FLORENCE	g.bacci@meyer.it
BAHR	ANGELA	INSTITUTE OF MEDICAL MOLECULAR GENETICS	SWITZERLAND	SCHWERZENBACH	bahr@medmolgen.uzh.ch
BALDUCCI	NICOLE	SANT'ORSOLA MALPIGHI HOSPITAL - BOLOGNA	ITALY	RIMINI	nicolebalducci@libero.it
BALOGH	ISTVAN	UNIVERSITY OF DEBRECEN	HUNGARY	DEBRECEN	balogh@med.unideb.hu
BAUWENS	MIRIAM	GHENT UNIVERSITY	BELGIUM	GHENT	miriam.bauwens@ugent.be
BEACH	ANNA	WESSEX CLINICAL GENETICS SERVICE	UK	SOUTHAMPTON	anna.beach@uhs.nhs.uk
BORG	ISABELLA	UNIVERSITY OF MALTA	MALTA	MSIDA	Isabella.borg@um.edu.mt
CAI	CAROLYN	COLUMBIA UNIVERSITY	USA	NEW YORK	cc3529@columbia.edu
CARVALHO	ANNA	CENTRO HOSPITALAR E UNIVERSITARIO DE COIMBRA	PORTUGAL	COIMBRA	aluisa.dcarvalho@gmail.com
CASALINO	GIUSEPPE	SCIENTIFIC INSTITUTE SAN RAFFAELE	ITALY	MILAN	casalino.giuseppe@hsr.it
CIPRIANI	VALENTINA	UCL INSTITUTE OF OPHTHALMOLOGY	ITALY	LONDON	v.cipriani@ucl.ac.uk
DE ZAEYTIJD	JULIE	GHENT UNIVERSITY HOSPITAL	BELGIUM	LOPPEM	julie.dezaeytijd@ugent.be
DEGLI ESPOSTI	SIMONA		ITALY	LONDON	simonadegliespsti@gmail.com

D'ESPOSITO	FABIANA	CEINGE	ITALY	NAPLES	desposito@ceinge.unina.it
ELLINGFORD	JAMIE	UNIVERSITY OF MANCHESTER	UK	MANCHESTER	jamie.ellingford@postgrad.manchester.ac.uk
GIFFNEY	CLAIRE	WEST MIDLANDS REGIONAL CLINICAL GENETICS SERVICE	UK	BIRMINGHAM	claire.giffney@bwhct.nhs.uk
HJORTSHOJ	TINA DUELUND	UNIVERSITY HOSPITAL OF COPENHAGEN	DENMARK	COPENHAGEN -	tina.duelund.hjortshoej@regionh.dk
HORVATH	PIROSKA	SEMMELWEIS UNIVERSITY	HUNGARY	BUPAPEST	b.horvath.piroska@gmail.com
KAMAKARI	SMARAGDA	OMMA INSTITUTE OF OPHTHALMOLOGY	GREECE	IRAKLION	kamakari@hotmail.gr
KAROLAK	JUSTYNA	POZNAN UNIVERSITY OF MEDICAL SCIENCES	POLAND	NOWE SKALMIERZYCE	justyna.karolak@gmail.com
KHAN	KAMRON	LEEDS INSTITUTE OF MOLECULAR MEDICINE	UK	HUDDERSFIELD	medknk@leeds.ac.uk
KOLLER	SAMUEL	INSTITUTE OF MEDICAL MOLECULAR GENETICS	SWITZERLAND	SCHWERZENBACH	koller@medmolgen.uzh.ch
LEE	WINSTON	COLUMBIA UNIVERSITY	USA	NEW YORK	W12355@columbia.edu
LEMBO	ANDREA	SAN GIUSEPPE HOSPITAL	ITALY	MILAN	andrealembo1984@hotmail.com
LIEW	GERALD	MOORFIELDS EYE HOSPITAL	UK	LONDON	gerald_liew@yahoo.com.au
LOSONCZY	GERGELY	UNIV. OF DEBRECEN	HUNGARY	DEBRECEEN	losigeri@gmail.com
MANITTO	MARIA PIA	SAN RAFFAELE HOSPITAL	ITALY	MILAN	manitto.mariapia@hsr.it
MEINDERT	JAN DE VRIES	UZ BRUSSEL	HOLLAND	ROTTERDAM	vrijei@xs4all.nl
MORARA	MARIACHIA RA	SANT'ORSOLA MALPIGHI HOSPITAL	ITALY	BOLOGNA	m_morara@hotmail.com
MULLANEY	BRENDAN	OXFORD GENETICS LABORATORIES	UK	OXFORD	brendan.mullaney@ouh.nhs.uk
MURRO	VITTORIA	CAREGGI HOSPITAL	ITALY	FLORENCE	vittoria.murro@unifi.it
NASSISI	MARCO	OPHTHALMIC HOSPITAL	ITALY	TURIN	m.nassisi@gmail.com
OLDAK	MONIKA		POLAND	ZIELONKA	monika.oldak@wum.edu.pl
PINAKAT	SAJISH		ITALY	BARI	segreteria@italianacongressi.it

PORTER	LOUISE	UNIVERSITY OF MANCHESTER	UK	MANCHESTER	louise.porter@postgrad.manchester.ac.uk
ROOMETS	EVA	TALLINN EYE CLINIC / UNIV. OF HELSINKI	ESTONIA	TALLINN	eva.roomets@itk.ee
ROSS	ALISON	NHS GRAMPIAN	UK	ABERDEEN	alison.ross3@nhs.net
SUJIRAKUL	THARIKARN	COLUMBIA UNIVERSITY	USA	NEW YORK	tsujirakul@gmail.com
SUZANI	MARTINA	SAN GERARDO HOSPITAL	ITALY	MONZA	martina.suzani@gmail.com
SYS	CELINE	UNIVERSITY GHENT	BELGIUM	SIJSEKLE	celinesys@hotmail.com
TAM	JOHNNY	ICFO - INSTITUTE OF PHOTONIC SCIENCES	SPAIN	CASTELLD EVELS	johnny.tam@icfo.es
TOMKINS	SUSAN	UNIVERSITY HOSPITALS BRISTOL	UK	BRISTOL	susan.tomkins@uhbristol.nhs.uk
VAN CAUWENBERGH	CAROLINE	CENTER FOR MEDICAL GENETICS - GHENT UNIV.	BELGIUM	GHENT	caroline.vancauwenbergh@ugent.be
WILLIAMS	DENISE	BIRMINGHAM WOMEN HOSPITAL	UK	BIRMINGHAM	denise.williams@bwhct.nhs.uk
XIE	YAJING	COLUMBIA UNIVERSITY	USA	NEW YORK	yx2187@columbia.edu

Participants - ABSTRACTS

Complexity of keratoconus genetics in Ecuadorian families

Justyna A. Karolak^{1,2}, Dorota M. Nowak^{1,2}, Jose A. Pitarque³, Andrea Molinari³, Bassem A. Bejjani⁴, Marzena Gajeczka^{1,2}

¹ Institute of Human Genetics, Polish Academy of Sciences, Poznan, Poland

² Department of Genetics and Pharmaceutical Microbiology, Poznan University of Medical Sciences, Poznan, Poland

³ Department of Ophthalmology, Hospital Metropolitano, Quito, Ecuador

⁴ Revemic Systems, Spokane, Washington

Keratoconus (KTCN) is a degenerative disorder of the eye associated with stromal thinning and protrusion of the cornea which results in altered refractive powers and loss of visual acuity. KTCN has a complex etiology and is caused by a combination of several factors. It has been suggested that environmental factors such as contact lenses, constant eye rubbing, atopy, and UV light exposure provoke oxidative stress leading to chronic corneal epithelial lesions and inflammation, and resulting in KTCN. However, familial inheritance, concordance between monozygotic twins, and association of KTCN with many known genetic syndromes strongly suggest that genetic factors are the dominant components in KTCN etiology.

KTCN without other ocular or systemic features was diagnosed in 18 Ecuadorian families. Genome-wide linkage analysis was performed in all families. In one family, novel locus for KTCN was mapped to 13q32, while in other families two loci were recognized at 2q13-q14.3 and 20p13-p12.2.

Candidate genes localized at identified KTCN chromosomal regions were examined in Ecuadorian families with PCR amplification and direct sequencing of all exons, promoters, UTRs, and intron-exon junctions. The sequencing analyses of genes from 13q32 locus have led to the identification of numerous sequence variants, including c.2262A>C and c.720+43A>G in *DOCK9*; c.2377-132A>C in *IPO5*, and c.1053+29G>C in *STK24* showing 100% segregation under a dominant model with KTCN phenotype in one large Ecuadorian family.

Analyses of genes from 2q13-q14.3 and 20p13-p12.2 loci have revealed c.214+242C>T in *ILIRN* and novel deletion c.2558+149_2558+203del54 in *SLC4A11* which were observed significantly more frequently in family members with KTCN.

Our results suggest involvement of these genes in KTCN etiology. However, identified variants are specific to the particular KTCN families only and cannot be recognized as causative in a general population. Lack of validation in larger numbers of affected families indicates significant genetic heterogeneity in KTCN. It has become clear that KTCN etiology may involve several genes and thus, further elucidation of the KTCN causes is needed.

Early dietary therapy in preventing progression of retinopathy in long-chain 3-Hydroxyacyl-CoA Dehydrogenase (LCHAD) deficiency caused by the homozygous G1528C mutation.

Roomets E(1), Kivelä T(2), Tyni T(3)

(1) *Department of Ophthalmology, Estonia*

(2) *Department of Ophthalmology, Helsinki*

(3) *Department of Pediatric Neurology, Helsinki*

Background: Long-chain 3-hydroxy-acyl-CoA dehydrogenase (LCHAD) deficiency belong to defects of mitochondrial fatty acid β -oxidation, the main energy producing pathway during prolonged exercise and fasting. Progressive pigmentary retinopathy is a characteristic feature of LCHAD deficiency, which may lead to visual impairment and disability in childhood or adolescence.

Methods: 13 Finnish patients with early diagnosis of LCHAD deficiency caused by the homozygous G1528C/Q510Q common mutation, were followed up to 20 years (median 6 y). An ophthalmologic examination was performed every 1-2 years.

Results: 11 out of 13 patients had mild pigmentary changes already in their first ocular exam (median age of 6 mos), while 2 had pale newborn retina. The initial fundus changes were most often pigment granularity in the posterior pole and hyperpigmented macula. In 12 patients, who had a good compliance of the dietary therapy, the pigmentary changes progressed only mildly or not at all, without any visual compromise. The only patient with an overall poor outcome, had progression of the retinopathy presenting atrophy in the posterior pole with relative sparing of the macula and far periphery.

Conclusion: The positive long-term outcome of 12 patients from 13 is in contrast with earlier reports of patients with a delayed start of the therapy who developed atrophic changes from an early age, which led to blindness by puberty. Our data are promising and suggest that the low-fat, high-carbohydrate diet started during the first months of life may delay or even halt the progression of the pigmentary retinopathy and possibly prevents the visual handicap.

Longitudinal tracking of parafoveal capillaries using adaptive optics reveals subclinical changes in a patient with type 1 diabetes

Johnny Tam, Ph.D.

Adaptive optics scanning laser ophthalmoscopy (AOSLO) can be used to noninvasively image and track photoreceptors, leukocytes, and capillaries. This case describes a 26 year old male patient diagnosed with Type 1 diabetes 20 years before his first visit with no history of hypertension or

hyperlipidemia [1]. The patient's left eye was followed over a period of 15 months during which there were a total of eight study visits. High resolution retinal videos were acquired in the parafoveal region using the AOSLO during four of the visits (AOSLO visits); spectral domain optical coherence tomography (SDOCT), fundus photography, HbA1c, Snellen visual acuity (VA), contrast sensitivity, and blood pressure measurements were taken during three of the visits (screening visits); during the study, the patient developed clinically significant macular edema in the contralateral eye and as a result fluorescein angiography (FA) was performed during one visit. Longitudinal assessment of AOSLO data revealed changes in the parafoveal capillaries, including microaneurysm formation and disappearance, formation of tiny capillary bends, and dropout of one capillary segment at the edge of the foveal avascular zone (FAZ) resulting in a small but significant increase in FAZ size. The screening visits found the patient to be clinically stable with severe non-proliferative diabetic retinopathy throughout the study. The FA confirmed that the AOSLO was able to generate images of the parafoveal capillary network. AOSLO images of capillaries were sharper than those seen by FA despite the fact that no contrast agents were injected for AOSLO imaging. These results establish that subclinical changes in the capillary network can occur over a period of 15 months even in the absence of clinically-detectable changes.

References

- [1] J. Tam, K.P. Dhamdhere, P. Tiruveedhula, B.J. Lujan, R.N. Johnson, M.A. Bearse, A.J. Adams, A. Roorda, "Subclinical Capillary Changes in Non Proliferative Diabetic Retinopathy," *Optometry and Vision Science* 89:692-703, 2012

Copy number analysis of *ABCA4* in Belgian patients with Stargardt reveals exon 20-22 deletion

Miriam Bauwens

Purpose:

Stargardt disease (STGD1) is one of the most frequent autosomal recessive retinal dystrophies, with a prevalence of 1/8000. Genetic testing for STGD1 is currently mostly limited to Sanger sequencing of the coding region of *ABCA4*. This approach leaves up to 30% of the STGD1 patients without a complete molecular diagnosis. In order to identify a second mutation, we performed quantitative PCR (qPCR) to screen for copy number variations (CNVs) in patients with one heterozygous or no *ABCA4* mutation.

Methods:

CNV screening comprised 50 qPCR assays, performed in 48 patients with one heterozygous or no mutation in *ABCA4* following *ABCA4* Asper chip and/or Sanger sequencing, and 27 controls (LC480, Roche). Breakpoint delineation was performed by additional qPCR assays, by sequencing using internal sequencing primers as well as next-generation sequencing (NGS) technology (Illumina, Miseq).

Results:

CNV analysis revealed a heterozygous deletion of exon 20-22 in a family segregating both STGD1 disease and autosomal recessive retinitis pigmentosa. The index patient and her affected brother were heterozygous for the splice site mutation c.5461-10C>T and this deletion. Using additional qPCR assays, followed by sequencing with internal sequencing primers and NGS of a patient specific junction PCR product, we could delineate the breakpoint regions to a total of

100 nucleotides. This deletion covers 4 kb, spans exons 20-22 and probably corresponds with a previously identified deletion of exons 20-22 in *ABCA4* (Maugeri et al. 1999). Analysis of the putative breakpoint regions reveals the presence of *Alu* elements, suggesting nonallelic homologous recombination as a possible mechanism underlying this deletion.

Additional patients with STGD1 or allied disorders with only one or no identified coding *ABCA4* mutation are being screened for this specific deletion using junction PCR. Haplotype analysis will be performed in additional deletion patients.

Significance: qPCR screening of the *ABCA4* coding region identified an *ABCA4* deletion of exon 20-22. To our knowledge only three genomic rearrangements of *ABCA4* have been described so far (Rozet et al. 1999, Maugeri et al. 1999, Yatsenko et al. 2003). Our and previous studies using high-resolution CNV analysis such as multiplex ligation-dependent probe amplification (MLPA) or qPCR suggest a very low prevalence of *ABCA4* deletions in STGD1, assuming the occurrence of non-coding variations or complex rearrangements in the remaining 30% of patients with only one or no coding mutation.