



**Meeting of the International Society for Genetic Eye  
Diseases & Retinoblastoma ISGEDR**

**Halifax, Nova Scotia, Canada  
August 6-8, 2015**

## **Welcome to Halifax and to *Your Meeting* ...**

Dear ISGEDR and other members of the Ophthalmic and Genetics communities,

We have the honor of welcoming you to the ISGEDR 2015 conference in Halifax, Nova Scotia, Canada. August is one of the best months to visit Nova Scotia and Halifax is a friendly seacoast city that has retained much of its historical charm. One can easily walk from the conference venue located at the edge of the downtown area to many historical sites, hotels, restaurants, shops and, of course, the lively waterfront.

The conference is held at Dalhousie University in one of the Faculty of Medicine's main auditoriums in the Sir Charles Tupper Medical Building (5850 College Street). We invite you to the Opening Reception at the Canadian Museum of Immigration at Pier 21, and the Gala Dinner at the Maritime Museum of the Atlantic. Both venues are located on the waterfront, and each offering a different perspective on Nova Scotia's rich maritime heritage locally and internationally. The museum's access will be reserved for the ISGEDR members during each event, and catering will be available on-site.

We look forward to what promises to be a very exciting conference, and we hope that you will have a memorable visit with us.

Johane Robitaille, M.D.  
*Meeting Chair, and Head of Local Organizing Committee*

David A. Mackey, M.D.  
*President, ISGEDR*

Elias I. Traboulsi, M.D.  
*Executive Vice-President, ISGEDR*

Brenda Gallie, M.D.  
*Member, Scientific Program Committee*

Kate Paton, M.D.  
*Member, Scientific Program Committee*

Ian MacDonald, M.D.  
*Member, Scientific Program Committee*

Arlene Drack, M.D.  
*Member, Scientific Program Committee*

Alex Levin, M.D.  
*Member, Scientific Program Committee*

Meghan Marino, M.S., L.G.C.  
*Member, Genetic Counseling Committee*

The International Society for Genetic Eye Diseases  
**ISGEDR**



**Mission Statement**

To bring together individuals interested in the field of genetic diseases  
of the eye and in Retinoblastoma

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To provide a forum for researchers in the field of genetic diseases  
of the eye to share information

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To promote international collaborations in the study of genetic diseases  
of the eye and in Retinoblastoma

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To disseminate scientific knowledge through international conferences  
and through its official publication, Ophthalmic Genetics

The International Society for Genetic Eye Disease & Retinoblastoma wishes to thank the following sponsors who have generously supported the 2015 ISGEDR Meeting

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## **Sincere Thanks to**

**Sandy Wong**

ISGEDR Coordinator,  
Cole Eye Institute  
Cleveland Clinic  
Cleveland, USA

**Sandi Leaf**

ISGEDR 2015 LOC  
Department of Pharmacology  
Dalhousie University  
Halifax, Canada

**Nadeem Traboulsi**

CME Department  
Cleveland Clinic  
Cleveland, USA

And the following Haligonian volunteers:

From the Orthoptics Clinical Vision Science Program, Dalhousie University

**Jessica Wood**

**Jenny Faries**

**Rebecca Fels**

**Lianne Esmores**

**Amr Zaki**

Resident, Department of Ophthalmology and Visual Sciences  
Dalhousie University  
Halifax, Nova Scotia

**Janet Brownell**

Administrative Assistant, Finances  
Department of Ophthalmology and Visual Sciences  
Dalhousie University  
Halifax, Nova Scotia

## Named Lecture Speakers for ISGEDR 2015

**Franceschetti Lecture:** *Elise Héon, Toronto, Canada*

**“Lessons Learned From Ocular Genetics: The Power of Phenotyping”**



**Elise Héon, MD, FRCS(C)**

*Professor, Department of Ophthalmology and Vision Sciences,  
University of Toronto*

Director of the Ocular Genetics Program

Mira Godard Chair in Vision Research

Associate Surgeon-in-Chief for Research

The Hospital for Sick Children

Toronto, Canada

former Chief of Ophthalmology (2003-2013)

Dr. Héon is a Clinician-Scientist in the field of Ocular Genetics, Director of the Eye Genetics Program and Senior Associate Scientist at The Hospital for Sick Children Research Institute in the program of Genetics and Genomics Biology. Dr. Héon carries both clinical and basic research projects. Her laboratory, supported by peer-reviewed grants, is involved in the genetic analysis of inherited eye disorders mostly retinal dystrophies.

Her clinical work focuses on the management of hereditary eye diseases, which include hereditary cancer (retinoblastoma) and other non-cancerous blinding conditions such as retinitis pigmentosa. Dr. Héon's role as Director of the Ocular Genetics program is to ensure that patients are provided with state of the art global care. This program is unique in Canada and part of only a few in the world. In addition to her numerous administrative activities, Dr. Héon has a busy teaching schedule dedicated to undergraduate, graduate and post graduate students as well as clinical and research Fellows. Through her research Dr. Héon works to better understand disease characteristics and mechanisms with the goal of improving outcome and quality of life of patients.

**François Lecture: Richard Weleber, Portland, USA**

**“Clinical Trials for Inherited Retinal Diseases: New Endpoints, Analyses”**



**Richard G Weleber, MD, FACMG**

*Professor, Department of Ophthalmology and  
Department of Molecular and Medical Genetics,  
Director of the Oregon Retinal Degeneration Center  
Casey Eye Institute of Oregon Health & Science University  
3375 SW Terwilliger Blvd, Portland, Oregon, USA*

Dr. Weleber is a Clinician-Scientist in the field of Ocular Genetics, retinal degenerations, visual electrophysiology, visual field methodology, and design and conduct of clinical trials. He founded the first visual electrophysiology service at OHSU in 1974 and was Director of the Visual Function Service for 39 years. He took two fellowships in Genetics—one at OHSU and the other at University of Colorado Medical Center. Since 1984, he has been the Director of the Oregon Retinal Degeneration Center at the Casey Eye Institute. He is board certified in both Ophthalmology and Clinical Genetics and was one of the Founding Members of the Ophthalmic Genetics Study Club, the International Society for Genetic Eye Disease, and the American College of Medical Genetics. Over the past 20 years, he has trained nine ophthalmologists in Ophthalmic Genetics, all of whom, following the Fellowship, accepted appointments at academic universities in the United States, England, and British Columbia.

With 40 years' experience in the study of inherited diseases of the retina, Dr. Weleber has evaluated, provided consultation, and cared for over 10,000 patients with a variety of ophthalmic genetic diseases. In his clinic he regularly identifies, screens and oversees the genetic testing of patients with retinal dystrophies, including retinitis pigmentosa and allied disorders. He has an international reputation in retinal electrophysiology and the evaluation of patients with retinal disease. He has a total of 197 peer-reviewed publications and numerous book chapters. Other major interests include improving the methodology for the evaluation of kinetic and static visual fields, volumetric measures of visual fields from 3D modeling of the Hill of Vision, and the development of clinical trial endpoints. He has received numerous honors including the Franceschetti Award/Lecture from the ISGEDR and the Adachi Award/Lecture from the ISCEV.

**Ellsworth Lecture: Junyang Zhao (China)**  
**“Retinoblastoma in China: Past, Present and Future”**



**Junyang Zhao, MD**  
*Senior Staff of Ophthalmology Department*  
Beijing Children's Hospital  
Capital Medical University  
Beijing, China

Dr. Junyang Zhao earned his MD from the Peking University in 1992 and completed his residency training at Beijing Tongren Hospital between 1992 and 1997. He became an attending ophthalmologist since then and received Master of Science Degree in 2004 from Capital Medical University. Dr. Junyang Zhao became the board member of Chinese Strabismus and Pediatric Ophthalmology Association in 2013.

Dr. Junyang Zhao started to focus on retinoblastoma in 2006 and has since treated more than 2000 patients. He developed collaborations with more than 20 hospitals all over China. He received around 300 new retinoblastoma cases each year and greatly improved the survival rate, saved many eyes for these patients. He moved to Beijing Children's Hospital in December 2014 and continues to work on retinoblastoma.



## Thursday August 6<sup>th</sup>

**8:00 Registration and breakfast**

**9:00 Welcome**

Drs. Robitaille and Mackey

**9:15 Official opening**

Faculty of Medicine Dalhousie University

**9:30 Ophthalmic Genetics Session 1 (Free papers)**

Moderator: Graeme Black

**9:30 IMPG1 AND IMPG2 CAUSE MACULAR VITELLIFORM DYSTROPHIES.** CHRISTIAN HAMEL, GAEL MANES, BEATRICE BOCQUET, ALMUNEDA AVILA-FERNANDEZ, SANDRO BANFI.

*Montpellier, France*

**Introduction:** Macular vitelliform dystrophies (VMD) comprise the juvenile Best disease, mostly caused by BEST1 mutations, and adult onset forms in which most cases, apart from rare RDS/PRPH2 mutations, are not genetically defined. We aimed at finding genes causing macular VMD and at describing their clinical features.

**Materials & Methods:** We performed gene mapping and whole exome sequencing in a large family with autosomal dominant VMD, and then screened 144 probands with various forms of macular dystrophy. In a second step, 49 probands with VMD were PCR sequenced for IMPG1 and IMPG2 mutations. Each patient had standard ophthalmic examination, color and autofluorescence fundus imaging, high resolution OCT scan and electro-oculogram.

**Results:** In the family with autosomal dominant VMD, we discovered that affected patients carried a heterozygous missense mutation (p.L238R) in IMPG1. Additional screening disclosed 2 other dominant families with this mutation, and 2 autosomal recessive VMD families with other IMPG1 mutations. Subsequently, we screened IMPG2 and found an autosomal dominant VMD family with a heterozygous missense mutation (p.C1077F). All patients had adult onset VMD and moderate loss of visual acuity (mean 20/40). Vitelliform deposits were moderately hyperautofluorescent and smaller than in Best disease, sometimes multifocal, with additional drusen like lesions. In OCT, the vitelliform deposits were localized above the RPE. The Arden ratio was normal or subnormal.

**Conclusions:** Mutations in IMPG1 and IMPG2 account for 8 % of VMD families negative for BEST1 and RDS/PRPH2, classified as adult-onset VMD and segregating either as dominant or recessive genetic conditions. IMPG1 and IMPG2 encode proteins of the mucin family, specific for the inter-photoreceptor matrix of the retina. They scaffold the outer segments of rods and cones and are probably important for the maintenance of their structural features, indicating that the interphotoreceptor matrix is, beside RPE, a critical actor in the pathophysiology of vitelliform dystrophies.

**9:42 PRESERVED VISUAL FUNCTION IN RETINAL DYSTROPHY DUE TO HYPOMORPHIC RPE65 MUTATIONS.** ANTHONY MOORE, SARAH HULL, RAJARSHI MUKHERJEE, ANTHONY G ROBSON, MICHEL MICHAELIDES, GRAHAM E HOLDER, ANDREW R WEBSTER. *San Francisco, California.*

**Introduction:** RPE65 related disease usually presents as a severe early-onset retinal dystrophy. In this study, 4 patients from 4 families with atypical, mild, recessive RPE65 related retinal dystrophy were investigated.

**Materials & Methods:** Detailed phenotyping included electrophysiological testing, color fundus photography, optical coherence tomography and fundus autofluorescence imaging. Bi-directional Sanger sequencing of all exons and intron-exon boundaries of RPE65 was performed on all reported patients and segregation confirmed in available relatives.

**Results:** Four patients, ascertained from the inherited retinal disease clinics, were screened for mutations in RPE65. All presented with nyctalopia in early childhood but demonstrated a mild phenotype with preservation of good central vision into adulthood. At last review, visual acuity in 3 patients ranged from 0.0-0.3 logMAR (20/20-20/40 Snellen) aged 18-26 years and in the 4th patient vision was 0.6 log MAR (20/80 Snellen) in the right eye and 1.0 logMAR (20/200 Snellen) in the left eye age 30 with symptoms of central vision loss only starting in his late 20s. One patient had extensive white dots throughout the retina reminiscent of fundus albipunctatus with electrophysiology demonstrating partial recovery of rod function after prolonged dark adaptation. Sanger sequencing confirmed bi-allelic RPE65 mutations in all patients. Two novel mutations were identified, including a premature terminating codon (PTC) c.1067dupA (p.Asn356Lysfs\*9) in a patient also carrying the established hypomorphic c.1543C>T (p.Arg515Trp) mutation. The other novel mutation was a missense c.433G>A (p.Ala145Thr) predicted to be tolerated on in silico analysis, found in the patient with a fleck retina phenotype and a previously reported PTC on her other allele, c.886dupA (p.Arg296Lysfs\*7).

**Conclusions:** RPE65 deficiency can cause mild disease in childhood with good visual acuity persisting into early adult life. A further case resembling fundus albipunctatus is described.

**9:54 GENOTYPE-PHENOTYPE CORRELATIONS IN CARRIERS OF PRPF31 MUTATIONS.** BIRGIT LORENZ, MONIKA ANDRASSI-DARIDA, WADIM BOWL, CHRISTOPHER FRIEDBURG, HANNO J. BOLZ. *Gießen, Germany*

**Purpose:** To describe the genetic and phenotypic features in five families with PRPF31 mutations.

**Materials & Methods:** Index cases from five families with a diagnosis of rod-cone dystrophy and macular edema underwent an extended ophthalmological examination including best corrected visual acuity (BCVA), Goldmann visual fields (GVF), fundus photography, optical coherence tomography with a Stratus 3 device (TD-OCT, Carl Zeiss Meditec AG, Jena) and a Spectralis device (SD-OCT, Heidelberg Engineering, Heidelberg, Germany), color vision testing (Lanthony Panel D15 saturated and desaturated), and electroretinography (ISCEV standard) with Ganzfeld (ERG, Nicolet Spirit, Nicolet Biomedical, Madison, WI) and multifocal testing (mfERG, Veris Science 5.1.D252, Electro-Diagnostic Imaging, Inc., Redwood City, CA). Also, fundus-controlled microperimetry (MP1), two-color threshold perimetry (2CTP), and full-field stimulus testing (FST) were performed on selected patients. The pedigree was recorded in all families, and DNAs of the index cases were subjected to Next Generation Sequencing (NGS) on

a panel of genes underlying retinal degenerative disorders. Available relatives underwent segregation testing of the molecular genetic results, and a CNOT3 polymorphism predicted to underlie the reduced penetrance in families carrying PRPF31 mutations was analyzed. A clinical examination was performed in the tested relatives.

**Results:** The inheritance pattern in the families was either inconclusive due to single cases (3/5 families) or could only be explained by reduced penetrance (2/5). Carriers of the underlying mutation developed an early onset rod-cone degeneration that could have been diagnosed as Retinitis pigmentosa on fundus photographs or were unremarkable as to function and/or morphology as previously described. The previously described CNOT3 polymorphism was identified in only two of five families but did not provide sufficient evidence to explain reduced penetrance in these families. Significant macular edema within the outer nuclear layer was present in all affected when a general loss of the photoreceptor layers was present. In early stages, a well preserved stratification without edema was present in the macula while peripheral stratification indicated already severe photoreceptor loss. This pattern could persist into the third decade. Edema also covered the inner nuclear layer but dissolved with progression. Progression did not correlate with the presence of the CNOT3 polymorphism.

**Conclusion:** PRPF31 is a reasonable candidate gene in single patients and families with rod-cone dystrophy and inconclusive inheritance patterns. Macular edema is a common feature in patients with advanced

**10:06 THE NATURAL HISTORY OF THE PROGRESSION OF ATROPHY SECONDARY TO STARGARDT DISEASE STUDIES (PROGSTAR): YEARLY PROGRESSION RATE OF ATROPHIC LESIONS IN THE RETROSPECTIVE STUDY.** RUPERT STRAUSS, ALEX HO, BEATRIZ MUNOZ, MOHAMED AHMED, SRINIVAS SADDHA. *Baltimore, Maryland*

**Introduction:** The multicenter ProgStar studies aim to characterize the natural history of Stargardt disease (STGD1) and to develop new outcome measures for clinical trials. The yearly rate of progression of STGD using the growth of atrophic lesions as measured by fundus autofluorescence (AF) imaging is the primary endpoint.

**Materials & Methods:** 251 patients were enrolled into the retrospective ProgStar study. FAF images (if available) were sent from the nine participating sites to a central reading center (Doheny Image Reading Center, CA) and areas of definitely decreased AF (DDAF), well-demarcated questionably decreased AF (WD-QDAF), and poorly demarcated questionably decreased AF (PD-QDAF) were outlined and quantified. Progression rates were estimated from linear mixed models with time as the independent variable. The models include random effects for the intercept, the slope of time, and the eye.

**Results:** 385 study eyes of 213 study participants enrolled in the retrospective study could be followed by available FAF imaging. Mean age at first visit was 29.7 years (observational time interval was 3.3 years, SD 1.5 years). At first visit, DDAF was present in 181 (47.0%) of the eyes, mean lesion size 2.4 (sd 2.8) mm<sup>2</sup>; WD-QDAF in 89 (23.1%), mean lesion size 1.2 (sd 1.2) mm<sup>2</sup>, and PD-QDAF in 237 (61.6%) mean lesion size 1.8 (sd 1.7) mm<sup>2</sup>. The estimated progression of DDAF at baseline was 0.54 mm<sup>2</sup> per year ( $p < 0.001$ ), the progression rate of WD-QDAF was - 0.200 (sd 0.05) mm<sup>2</sup> and the one of PD-QDAF 0.10 (sd 0.03)mm<sup>2</sup>. The estimated cumulative incidence of DDAF areas in the 204 eyes without involvement at first visit was 44.6% by the end of the follow-up time.

**Conclusions:** FAF may serve as a monitoring tool for interventional clinical trials in STGD that aim to slow down disease progression. However, additional outcome measures may be needed for subtypes of patients showing lesions of WD-QDAF and will be further evaluated in the ProgStar studies.

**10:18 THE IMPACT OF FOUNDER EFFECT ON GENOTYPIC-PHENOTYPIC CORRELATIONS IN THE FIRST COMPREHENSIVE SCREENING OF AUTOSOMAL DOMINANT RETINITIS PIGMENTOSA (ADRP) GENES IN THE FRENCH-CANADIAN POPULATION.** RAZEK GEORGES COUSSA, CHRISTINA CHAKAROVA, FARES ANTAKI, AYESHA KHAN, IRMA LOPEZ, HUANAN REN, NAUSHIN WASEEM, KUNKA KAMENAROVA, SHOMI S BHATTACHARYA, ROBERT K KOENEKOOP. *Montreal, Canada.*

**Introduction:** The French-Canadian population of Quebec is a well-known founder population due to common ancestry from ~8500 French forefathers. The genetics of retinitis pigmentosa (RP) in Quebec is still incomplete despite intense studies. The purposes of our study were to quantify the prevalence of adRP mutations, characterize its associated phenotypes and establish a link between Quebec and France adRP mutations.

**Materials & Methods:** DNA from 60 probands from adRP French Canadian families was isolated by standard methods. All coding exons and flanking intronic areas of the selected adRP genes were PCR amplified, purified and sequenced. *in silico* bioinformatic tools were used to assess the effect of novel mutations. Sanger sequencing was used to confirm co-segregation of pathological within the families. Phenotypes were analysed by best-corrected visual acuity, slit lamp biomicroscopy, funduscopy, Goldmann visual fields (GVF), electro-retinography (ERG), fundus autofluorescence (FAF) and Optical coherence tomography (OCT).

**Results:** Among the 60 studied families, we successfully settled the causal mutation in 24 adRP patients (40% of cohort). 6 known genes harboured the 24 mutations: eleven (46%) in RHO, 4 mutations (17%) in SNRNP200, 3 mutations (12.5%) in PRPH2/RDS, 3 mutations (12.5%) in TOPORS, 2 mutations (8%) in PRPF31 and 1 mutation (4%) in IMPDH1. We report six novel and present the genotype-phenotype characteristics of five identified in RHO (p.R135G), PRPF31 (p.R288W), IMPDH1 (p.Q318H), SNRNP200 (p.V708I) and TOPORS (p.H889R).

**Conclusions:** To date, our study provides the first comprehensive screening of adRP genes in the French Canadian founder population of Quebec. Our prevalence of known adRP genes is 40%, which is lower in other adRP populations around the world. This emphasizes the importance of founder effect and the uniqueness of the French Canadian population. Our findings are key in establishing the genotypic-phenotypic spectrum of RP in Canada and expanding the current understanding of childhood blindness and identifying new causal genes, new proteins and novel retinal pathways.

**10:30 PNPLA6-OPATHIES: AN EMERGING SPECTRUM OF CONGENITAL, CHILDHOOD, AND ADULT NEURODEGENERATIVE CONDITIONS.** ROBERT B. HUFNAGEL, SARAH HULL, ROBERT A. SISK, GAVIN ARNO, CORINNE STOETZEL. *Cincinnati, Ohio.*

**Introduction:** Oliver-McFarlane syndrome is a rare disorder with a phenotypic triad of congenital trichomegaly, chorioretinal atrophy, and hypopituitarism. Laurence-Moon syndrome has a clinical presentation similar to Oliver-McFarlane syndrome, including chorioretinopathy and pituitary dysfunction, though with childhood onset of ataxia, peripheral neuropathy, and spastic paraplegia. We and others recently demonstrated that mutations in

PNPLA6 result in these congenital neurodegenerative disorders due to dysfunction of the encoded protein, Neuropathy Target Esterase (NTE). Previously, PNPLA6 mutations have also been associated with similar conditions with later onset, namely Boucher-Neuhauser syndrome, Gordon-Holmes syndrome, and Spastic Paraplegia type 39. Here, we discuss the mechanistic findings and the natural history of chorioretinopathy in Oliver-McFarlane and Laurence-Moon syndromes in our patients.

**Materials & Methods:** DNA was analyzed for PNPLA6 mutations by Sanger sequencing or by whole exome sequencing with Sanger confirmation. Retinal phenotypes were specifically analyzed by visual acuity, color vision testing, fundus photos, fundus autofluorescence, optical coherence tomography (OCT), visual fields, and electroretinography (ERG). Brain MRI and hormone levels were obtained to assess hypopituitarism and spinocerebellar phenotypes.

**Results:** Disease-causing variants in PNPLA6 included missense, frameshift, and splice site mutations, the majority of which alter the esterase domain. Chorioretinal degeneration was typically diagnosed in the first five years of life. Thyroid and growth hormone replacement during improved developmental and growth delays when initiated during childhood. However, vision loss was progressive and devastating. Chorioretinal atrophy with preserved macular and peripapillary pigment was noted in all patients. Additionally, patients typically had constricted visual fields, macular atrophy on OCT, and reduced or absent cone and rod activity on ERG.

**Conclusions:** Oliver-McFarlane syndrome is caused by PNPLA6 mutations that result in early and severe loss of NTE function. Chorioretinopathy is characteristic of this condition and is also present in PNPLA6-associated Laurence-Moon and Boucher-Neuhauser syndromes. The progression and severity of vision loss varied among our patients. Ongoing studies will further focus on natural history, genotype-phenotype correlations, and the relationship between NTE activity and disease prognosis.

**10:42 AUTOSOMAL RECESSIVE MICROCEPHALY WITH CHORIORETINOPATHY (MCMR) AND MUTATIONS IN TUBGCP4, ENCODING A MEMBER OF THE GAMMA-TUBULIN RING COMPLEX GAMMA TURC.** HELENE DOLLFUS, SOPHIE SCHEIDECKER, CHRISTELLE ETARD, LAURENCE HAREN, SARAH HULL. *Strasbourg, Germany.*

**Introduction:** Microcephaly and chorioretinopathy is a well known clinical entity currently being deciphered on a molecular level with a growing number of genes identified thus unveiling the biological pathways but also helping in genetic counselling and care for families.

**Materials & Methods:** We have performed whole exome sequencing in a panel of families with either sporadic or recurrent microcephaly and retinopathy.

Validation of the candidate gene was performed with zebrafish assays as well as in vitro investigations on patients cells.

**Results:** On one family and independently on another family compound-heterozygous mutations in TUBGCP4 were found. Subsequent Sanger sequencing was performed on a panel of individuals from 12 French families affected by microcephaly and ophthalmic manifestations, and one other individual was identified with compoundheterozygous mutations in TUBGCP4. One synonymous variant was common to all three families and was shown to induce exon skipping; the other mutations were frameshift mutations and a deletion. TUBGCP4 encodes g-tubulin complex protein 4, a component belonging to the g-tubulin ring complex (g-TuRC) and known to regulate the nucleation and organization of microtubules. Functional analysis of

individual fibroblasts disclosed reduced levels of the  $\gamma$ -TuRC, altered nucleation and organization of microtubules, abnormal nuclear shape, and aneuploidy. Moreover, zebrafish treated with morpholinos against *tubgcp4* were found to have reduced head volume and eye developmental anomalies with chorioretinal dysplasia.

**Conclusions:** The identification of TUBGCP4 mutations in individuals with microcephaly and a spectrum of anomalies in eye development, particularly photoreceptor anomalies, provides evidence of an important role for the  $\gamma$ -TuRC in brain and eye development.

#### **10:54 NOVEL MUTATIONS IN KIF11 GENE IN JAPANESE PATIENTS WITH FEVR AND MLCRD .**

HIROYUKI KONDO, ITSUKA MATSUSHITA, EIICHI UCHIO, SHUNJI KUSAKA. *Kitakyusyu Japan*

**Introduction:** We report the characteristics of 8 Japanese patients with familial exudative vitreoretinopathy (FEVR) or microcephaly, lymphedema, and chorioretinal dysplasia (MLCRD) who had mutations in the KIF11 gene.

**Materials & Methods:** Ophthalmic and systemic evaluations were performed on eight patients with FEVR or MLCRD. Two patients were probands of familial cases and six patients were sporadic cases. DNA was extracted from the patients, and whole exome sequence followed by confirmatory Sanger sequence analyses were performed on seven patients. Direct Sanger sequence on each exon of the KIF11 gene was performed in one patient. Prior to the study, all patients had been found not to carry mutations in known FEVR-causing genes.

**Results:** Whole exome sequence showed two familial and four sporadic patients had three novel and two known mutations in the KIF11 gene (p.S235C, p.Q290\*, p.R387\*, p.L847fs, and p.T926fs). In addition, exome sequence revealed that one patient had a genomic loss of heterozygosity (LOH) of 1.3 Mb on chromosome 10 surrounding the KIF11 gene suggestive of a large deletion. Direct Sanger sequencing revealed that one sporadic patient had a novel frame shift mutation (p.D579fx). The severities of their vitreoretinopathies were: bilateral leukocoria (N=1); leukocoria in one eye and a falciform retinal fold in the other eye (N=1); bilateral falciform retinal folds (N=5); and a falciform retinal fold in one eye and FEVR-like retinal vascular anomaly with choroidal atrophy (N=1). Systemic anomalies included microcephaly with or without mental retardation (N=5). No patients had lymphedema.

**Conclusions:** Our data add evidence that mutations in the KIF11 gene can cause the spectrum disorders, FEVR and MLCRD. Mutations in the KIF11 gene account for high percentage in patients who do not carry known gene mutations responsible for FEVR. The patients with KIF11 mutations may have more severe vitreoretinopathy than FEVR patients with mutations in other FEVR-causing genes. The degree of microcephaly is highly variable and tends to be overlooked.

#### **11:06 THE EXTENDED MOLECULAR HETEROGENEITY OF FAMILIAL EXUDATIVE**

**VITREORETINOPATHY AND MICROCEPHALY.** SARAH HULL, GAVIN ARNO, PIA OSTERGAARD, LOUISE BICKNELL, CAROL-ANNE MARTIN. *London, United Kingdom*

**Introduction:** Familial exudative vitreoretinopathy (FEVR) is rarely associated with microcephaly. The aim of this study was to demonstrate the range of genetic heterogeneity and the potential benefits of a molecular diagnosis.

**Materials & Methods:** A series of 13 children from 10 families with a clinical diagnosis of FEVR and microcephaly were recruited. Molecular investigations included candidate gene Sanger

sequencing and whole exome sequencing (WES). Clinical investigations included retinal imaging and electrophysiology.

**Results:** Analysis has so far identified likely disease-causing variants in 5 out of 10 families under investigation. Two families have LRP5 related disease. The first, investigated with autozygosity mapping then candidate gene Sanger sequencing, has a novel homozygous splice mutation, c.4112-3C>G that segregated with disease. Due to the molecular diagnosis, bone density investigations were performed with a normal result. The 2nd family was identified by Sanger sequencing to have a heterozygous novel missense variant c.3914G>A (p.Cys1305Tyr) in the affected child and the mother who had previously had a normal dilated fundus examination. Subsequent Optos widefield fundus fluorescein angiography in the mother confirmed abnormal peripheral retinal perfusion. A bone density scan in the affected 5 year old child was abnormal with a z-score of -2.1 indicating osteoporosis and providing further evidence for the causality of the molecular finding. A male patient presented in infancy with reduced vision and was found to have a retinal fold in the left eye with areas of chorioretinal atrophy in the right eye. Electroretinogram (ERG) identified a rod-cone dystrophy. Screening of KIF11 by Sanger sequencing identified a novel heterozygous premature truncating codon, c.2910\_2914del (p.Glu970Aspfs\*17). A 6 month-old male patient with bilateral retinal folds, and a rod-cone dystrophy with enhanced S-cone-like features on ERG was investigated with WES as were both parents. This identified a novel, de novo nonsense mutation, c.247C>T (p.Arg83\*), in KIF11 as the likely causative mutation. Finally, a family of 2 children with learning difficulties, abnormal peripheral retinal vasculogenesis and rod-cone dystrophy were investigated with candidate gene sequencing. They were found to have bi-allelic novel, mutations in TUBGCP6, c.2066-6A>G and c.4485-21A>C with RNA analysis confirming abnormal splicing. Of the 5 unsolved families, 4 have had investigation by Sanger sequencing of KIF11 followed by WES, with no causative variants identified to date. The fifth family is undergoing whole genome investigation.

**Conclusions:** Molecular diagnosis has been achieved in half of the families investigated. This has enabled patient-specific care with targeted investigations and accurate family counseling.

### **11:18 PHENOTYPIC SPECTRUM AND MOLECULAR DIAGNOSIS OF FAMILIAL EXUDATIVE VITREORETINOPATHY (FEVR) AND RELATED CONDITIONS IN AN INTERNATIONAL DATABASE.**

JOHANE ROBITAILLE, KARIN WALLACE, JILL BEIS, ROXANNE GILLETT, MARISSA LEBLANC, DANIEL GASTON, MATHEW NIGHTINGALE, MICHAEL P MACKAY, CHRISTINE MCGILLIVRAY, CHRISTOPHER MCMASTER, KAREN BEDARD. Halifax, Nova Scotia Canada

**Introduction:** To describe the phenotypic spectrum of disease caused by FEVR gene mutations and the role of molecular diagnosis in differentiating FEVR from related conditions.

**Materials & Methods:** Individuals with a diagnosis of FEVR, Coats disease, congenital retinal detachment, childhood onset retinal folds, retinopathy of prematurity (ROP) and persistent fetal vasculature (PFV) along with their relatives were invited to participate. Clinical data and DNA were collected from participants since 1998 from the clinical practices of ophthalmologists and clinical geneticists internationally. Direct automated sequencing of the coding regions of the known FEVR genes, NDP, FZD4, LRP5, TSPAN12 and ZNF408, and/or whole exome sequencing was conducted to identify disease-causing mutations.

**Results:** 92 probands were enrolled since 1998: 76 with a diagnosis of FEVR and 16 with Coats disease. The mutation detection rate in the FEVR group was 38% (29/76) with the greatest proportion affecting FZD4 (17%) followed by LRP5 (13%) and TSPAN12 (3%). Whole exome sequencing identified a mutation in KIF11 in one pedigree. Direct sequencing of the coding regions of the KIF11 gene in probands diagnosed with FEVR and without a known FEVR gene mutation yielded a total of four probands (5%) with a KIF11 mutation: these probands were subsequently diagnosed with microcephaly and chorioretinal dysplasia. Eight probands were heterozygous for an LRP5 mutation that was previously identified in patients with osteoporosis-pseudoglioma syndrome, a recessive condition that has not been reported to manifest features of FEVR in heterozygous carriers. A FZD4 missense mutation in non-conserved areas of the gene was found in 2/71 (3%) infants with severe ROP requiring treatment or causing blindness in a multi-centre case-control study. No FZD4 mutation was found in patients with unilateral or bilateral Coats disease. At least three patients from two pedigrees with a FZD4 mutation were initially diagnosed with possible or likely PFV.

**Conclusions:** Significant phenotypic and molecular overlap exists between FEVR and other conditions that affect the development of the retinal vasculature. A molecular diagnosis can help distinguish FEVR from disorders that are associated with systemic abnormalities. The mutation detection rate in FEVR suggests that other genes remain to be identified.

**11:30**      **Break/Poster Viewing**

**12:00**      **Franceschetti Lecture: Elise Héon, Toronto, Canada**

Lessons learned from Ocular Genetics: the power of phenotyping  
Introduction by Elias I. Traboulsi

**12:45**      **Lunch/Poster Viewing**

**13:45**      **Retinoblastoma Session 1 (Free papers)**

**Moderator: Bruce Crooks**

**13:45 GOOGLING DR GOOGLE: LEUKOCORIA, RETINOBLASTOMA AND THE WORLD WIDE WEB.**  
SANDRA STAFFIERI, ALEX HEWITT, LISA KEARNS, DAVID MACKEY. *Melbourne, Australia*

**Introduction:** Leukocoria is the most common presenting sign for retinoblastoma and cataract. Often the leukocoria is seen by the parent in family photographs or with the naked eye. Delay in diagnosis for retinoblastoma is common and can result in further advancement of the disease with adverse outcomes. All published reports on delayed diagnosis of retinoblastoma recommend improved education for health practitioners and the general community alike of the potentially sinister sign of leukocoria. This project aimed to determine the public perception of leukocoria as seen in photographs as well as examine the free text search terms used by them to seek more information.

**Materials & Methods:** An interactive web-based questionnaire incorporated two photographs of a child with leukocoria. Participants were asked to search the internet to determine a



diagnosis and action plan. The most commonly used search terms were recorded along with the websites visited. Word of mouth, posters and social media were utilized to promote participation in the project.

**Results:** The web-based questionnaire attracted 1665 respondents predominantly from Australia. Facebook advertisement yielded the best participation rate. Ninety-four percent of respondents were female. The age of respondents ranged from 15-83 years with a mean of 38.6 years. Fifty- eighty percent of respondents had children. Eighty-four percent of respondents noted an abnormality in the photos presented in the questionnaire. A combination of individual words or phrases were used by respondents to seek more information. Individually or as part of a phrase, the most commonly used search terms were white, pupil and "ophoto". Using these search terms a variety of appropriate websites or links to media articles were reached providing useful information. Following the web-based searches, 49% correctly identified retinoblastoma as the most likely diagnosis, conversely 4% did not notice any abnormality at all and were not concerned.

**Conclusions:** Retinoblastoma is the most commonly occurring intraocular tumour in children. With leukocoria being the most common presenting sign, it is often seen in photographs "all too often retrospectively. Raising awareness of leukocoria and retinoblastoma for the general public and health professionals alike should improve early referral for children with retinoblastoma. Being aware of common search terms used by parents will allow optimisation of search engines and retinoblastoma web sites to assist earlier diagnosis and referral.

**13:57 BUILDING A LEARNING HEALTH SYSTEM FOR RETINOBLASTOMA.** BRENDA GALLIE, TRAN TRUONG, JUSTIN LIU, YULIYA GAVRYLYUK. *Toronto, Ontario*

**Introduction:** Retinoblastoma is a complex disease with multiple recurrent treatments and multiple tumors in two eyes. Institutional health records are not well suited to these details. Clinical trials and research have been rarely completed in retinoblastoma, resulting in a low quality of evidence for care.

**Materials & Methods:** eCancerCareretinoblastoma (eCCRB) was developed over 15 years to capture specific data necessary for care of patients with retinoblastoma. In one institution, eCCRB has been used as a summary of details of care. Version 2 of eCCRB is now being deployed on a "cloud" server with enhanced features.

**Results:** More than 400 retinoblastoma patients have been managed with eCCRB as a point-of-care database summarizing overall care. The Disease-specific electronic Patient Clinical Timeline (DePICT) has been validated to convey at a glance, an accurate understanding of the patient for care-givers and parents. eCCRB v2 collects clinical details and automatically classifies eye(s) by 4 different classification schemes. eCCRB v2 has enhanced retinal drawing capability with vector coordinates; each subsequent visit pulls data from the past visit for modification, making the job of accurate records easy. eCCRB has been used in Kenya as a registry. To share this resource world-wide and support global research, eCCRB is now in demo phase on a cloud-based server. When privacy and security impact assessments are complete, with full consent from care-givers and patients, retinoblastoma treatment centres world-wide will be able to use eCCRB in clinical care with data only identified to those in the circle of care. With collaborative agreements in place, the de-identified data will be used as the core of a learning health system.

Real outcomes with all clinical details will be mined in real time, in support of evidence-based management of the next patient.

**Conclusions:** Significance: eCCRB provides a flexible tool to both enhance care and capture high quality complex data for research.

**14:09 WHAT DIFFERENCE DOES NEXT GENERATION SEQUENCING MAKE TO RETINOBLASTOMA GENETIC TESTING?** HILARY RACHER, SHARLENE KOROSCIL,CRYSTAL D'SILVA,XUE WU,CHAO ZHANG,XIAOFANG HUANG,FRANNY JEWETT,YANG SHAO,BRENDA GALLIE. *Toronto, Ontario*

**Introduction:** Next generation sequencing (NGS) is being employed in a large number of molecular genetic laboratories around the world. At high volumes of patient samples and genes studied, NGS is a cost-effective and efficient tool for deeper interrogation of sequencing-detectable variants. For *RB1*, focus has been on the NGS potential for discovery of low-level mosaic variants. We report the increased sensitivity of NGS on our bilateral retinoblastoma genetic test and more broadly, its impact on cost effectiveness of clinical genetic testing. Cost-effectiveness of genetic testing in retinoblastoma is well known (Noorani et al 1996 PMID: 8755916).

**Materials & Methods:** DNA from bilateral probands with no *RB1* mutation found at Impact Genetics was targeted using custom designed biotinylated probes for hybridization based capture of the *RB1* gene coding sequence, flanking intronic regions and the core promoter. NGS was performed using the Illumina MiSeq. Variants were confirmed by allele-specific PCR. The cost-effectiveness of NGS and conventional technologies were compared for retinoblastoma genetic testing by decision analytic modeling using Canadian standard of care and costs.

**Results:** All bilateral retinoblastoma cases are expected to carry a germline mutation in the *RB1* gene. However, extensive genetic analysis reveals no *RB1* mutation in DNA of 3.4% of 757 bilateral retinoblastoma patient blood samples tested in our laboratory by conventional methods (Sanger sequencing, copy number detection and allele-specific PCR for 11 recurrent mutations). We report a 0.4% increase in test sensitivity after very deep (>400x) NGS sequencing. To address the cost effectiveness of NGS, we summarized assay cost and overall test sensitivity of NGS only (becoming more common around the world), Sanger sequencing and copy number only (most common in North American labs), and our lab's methods with and without NGS. Sensitivity analysis demonstrated that the incremental cost effectiveness ratio (ICER) was most influenced by test sensitivity followed by number of relatives and cost of testing. Our analysis demonstrates that NGS in conjunction with our conventional methods enhances sensitivity for bilateral retinoblastoma. However, low sensitivity of NGS alone results in a high false negative rate for unilateral patients, incurring high costs of health care.

**Conclusion:** NGS is effective to moderately increase the *RB1* mutation detection rate in bilateral patients, and in combination with conventional methods, is cost effective.

**14:21 MANAGEMENT AND OUTCOMES OF RETINOBLASTOMA IN THE REPUBLIC OF IRELAND.** WE FONG SIAH, BERNADETTE LANIGAN, BRENDA GALLIE, MICHAEL O'KEEFE. *Republic of Ireland*

**Introduction:** Retinoblastoma (RB) cases in the Republic of Ireland are primarily managed at the Children's University Hospital, Dublin by our senior author (M.O.K.). Telemedicine was utilized and cases discussed with RB expert (B.G.) especially in the later series. We report our experience in the management of RB, outcomes and any associated complications.

**Materials & Methods:** Retrospective review of consecutive cases of RB between 1990 and 2014.

**Results:** There were 50 patients with unilateral disease (30 males; mean age  $\hat{\pm}$  SD, 27.9  $\hat{\pm}$  14.4 months) and 13 patients with bilateral disease (8 males; median age, 12 months, range 2  $\hat{\pm}$  37 months), 2 of which were triphasic RB. International Classification of Retinoblastoma grading: Unilateral RB (group A, n = 1 (2%); group B, n = 1 (2%); group C, n = 2 (4%); group D, n = 13 (26%); group E, n = 29 (58%); missing info, n = 4 (8%)) and bilateral RB (more affected eye: group C, n = 3 (23%); group D, n = 4 (31%); group E, n = 6 (46%); fellow eye: group A, n = 2 (15%); group B, n = 4 (31%); group C, n = 3 (23%); group D, n = 4 (31%)). For unilateral RB, enucleation was performed in 45 cases (90%) while the remaining cases received focal treatment (photocoagulation and/or cryotherapy alone, n = 4; intravitreal and intra-arterial melphalan (treated elsewhere), n = 1). Of these, 18 patients (36%) received adjuvant systemic chemotherapy. For bilateral RB, 9 patients (69%) had unilateral enucleation with 2 (15%) requiring subsequent enucleation of the other eye while the remaining 4 cases (31%) had successful preservation of both eyes. All bilateral RB cases received systemic chemotherapy. Mortality rate was 3.2% (1 case of unilateral RB treated with enucleation alone only; 1 case of triphasic RB). Overall, a total of 56 enucleations were performed. Socket-related complications were evident in 25 patients; 8 (14%) conjunctival dehiscence alone, 15 (27%) exposed implant (scleral patch graft, n = 10; implant exchange, n = 4; dermis fat graft, n = 1), 1 implant extrusion requiring implant exchange and 1 post-enucleation socket syndrome.

**Conclusions:** Given the rarity of RB and the small population in this nation, our study shows the successful management of RB with utilization of telemedicine. We recognized a high rate of socket-related complications largely attributed by poor-fitting prosthetic eye. We have now changed our practice to provide customized, tailored-made prosthetic eye for each patient.

## 14:45 Ophthalmic Genetics Session 2 (Free papers)

Moderator: David Mackey

**14:45 NOVEL FOXC1 MUTATIONS WITHIN THE DNA-BINDING FORKHEAD DOMAIN RESULT IN A CONGENITAL OCULAR PHENOTYPES OF AXENFELD-RIEGER SYNDROME.** HANNAH L. SCANGA, BHARESH CHAUHAN, ANAGHA MEDSINGE, SUNEETA MADAN-KHETARPAL, KEN K. NISCHAL. *Pittsburgh, PA.*

**Introduction:** Axenfeld-Rieger syndrome (ARS) is characterized by anterior segment dysgenesis and, in some cases, a range of extra-ocular features. Iris and trabecular meshwork abnormalities associated with ARS contribute to a 50% lifetime risk for glaucoma, which tends to be a juvenile or adult-onset condition(1). In at least 40% of cases, ARS is associated with a FOXC1 or PITX2 mutation(2). Here, we report three unrelated patients with novel FOXC1 mutations who presented with congenital corneal clouding and glaucoma associated with ARS.

**Materials & Methods:** Retrospective case review of three unrelated infants, two females and one male, referred for ophthalmic examination.

**Results:** Each patient presented before 2 months of age with severe, bilateral, congenital corneal clouding and intraocular pressures requiring surgical intervention. In two cases, further ocular examination revealed variable anterior segment abnormalities associated with ARS, including posterior embryotoxon, iris hypoplasia, and corectopia. In the third case, there was severe aniridia. Systemic examinations were significant for umbilical hernia and dysmorphic facial features in a single patient. Sequencing of FOXC1 and PITX2 revealed three novel FOXC1 mutations, specifically c.289\_299del11 (p.L97PfsX205), c.456C>G (p.W152C), and c.385A->\_387del. Family histories were non-contributory and parental testing revealed that the mutation appeared to be de novo in each case. All three mutations are located within the DNA-binding forkhead domain (FHD) of the FOXC1 protein.

**Conclusions:** Mutations within the DNA-binding FHD of FOXC1 result in a loss-of-function and affect protein-protein interactions, with the potential to cause both ocular and systemic features (3). In our cohort, each FOXC1 mutation was located within the FHD, but only the nonsense mutation was associated with extra-ocular features of ARS. This may suggest a correlation between retained FOXC1 protein function and the resultant phenotype. Furthermore, all three novel mutations resulted in severe, congenital ocular phenotypes, which is atypical for ARS. In summary, these three cases further demonstrate the importance of the FOXC1 DNA-binding FHD and its association with the development of ocular and extra-ocular features of ARS.

#### **14:57 FOXC1 MUTATION WITH SIGNIFICANT POSTNATAL IRIS GROWTH AND MODULATION.** MERINA THOMAS. BEHRAD Y MILANI, IRENE H MAUMENEE, JAVANEH ABBASIAN. *Chicago, Illinois*

**Introduction:** The term anterior segment dysgenesis comprises a complex of disorders resulting in corneal, lens or whole eye abnormalities with detrimental visual consequences. The most commonly mutated genes are PITX2, FOXC1 and PAX6. The natural history is one of progressive decline of visual function due to development of a corneal pannus, corneal perforation, glaucoma, progressive iris atrophy or phthisis. We are presenting here a child with major postnatal iris growth and partial normalization of anatomy in the absence of surgical intervention

**Materials & Methods:** Longitudinal observation of a child with two distinct anterior segment phenotypes: anterior segment dysgenesis and aniridia in the right and left eye respectively. DNA sequence analysis of patient and parents a novel mutation in FOXC1

**Results:** The eye with anterior segment dysgenesis underwent progressive normalization of the anterior segment over a five year period. More recently extension of previously observed peripheral nasal iris atrophy was observed in the presence of further iris extension and pupillary displacement towards temporal. The eye with aniridia remained unchanged over this period. The most recent visual acuities are 20/60 and FC at 10 inches with a hypermetropic correction. He has intermittent jerk nystagmus. The intraocular pressures are controlled. Detection of a novel mutation in FOXC1.

**Conclusions:** Growth potential of the iris in patients with anterior segment dysgenesis may be preserved during a postnatal period of unknown length.

**15:09 FERRITIN LIGHT CHAIN GENE MUTATION IN A LARGE AUSTRALIAN FAMILY WITH HEREDITARY HYPERFERRITINEMIA-CATARACT SYNDROME.** SEYHAN YAZAR, MARIA FRANCHINA, KATHRYN BURDON, DAVID A MACKAY. *Nedlands WA, Australia*

**Introduction:** Hereditary hyperferritinemia-cataract syndrome (HCCS) is an autosomal dominant Mendelian disorder characterised by early onset cataracts and elevated levels of serum ferritin in the absence of iron overload. Multiple HCCS causing mutations were identified in the 5'UT non-coding region of the ferritin light chain (FTL) gene in family studies. We present an Australian family with 10 affected members spanning three generations.

**Materials & Methods:** DNA was prepared from blood and saliva samples received from the family members. The complete sequencing of the iron-responsive element (IRE) of the FTL gene was analysed using bi-directional genomic DNA sequencing.

**Results:** We identified a heterozygous single nucleotide substitution (c.-167 C>T) in the index case and five affected family members (logarithm of the odds score [Z]=3.61, recombination distance[ $\hat{\theta}$ ]=0). The age of the cataract onset was varied between the affected individuals. All individuals were previously diagnosed with high ferritin levels.

**Conclusions:** This is the first report of this mutation in a large family with multiple affected individuals. This study raises the possibility that identification of HCCS mutations may be an effective means of disease detection and facilitate appropriate genetic counselling.

**15:21 CONGENITAL EYE MOVEMENT DISORDERS: IMPROVING PHENOTYPIC DESCRIPTIONS AFTER GENETIC ANALYSIS- ANOTHER LOOK AT MOEBIUS SYNDROME.** DARREN OYSTRECK, SARAH MACKINNION, CAROLINE ANDREWS, WAI-MAN CHAN, DAVID HUNTER. *HALIFAX, NOVA SCOTIA, CANADA*

**Introduction:** This past decade has seen a change in the classification of congenital eye movement disorders following the genetic elucidation of many forms. This group is now referred to as the Congenital Cranial Dysinnervation Disorders (CCDDs), a term used to highlight that the primary underlying problem is maldevelopment of innervation of the extra ocular muscles. Prior to this these disorders were distinguished by phenotypic features alone. As genetic distinctions became available phenotypic patterns were modified to encompass the main features of these conditions. Moebius syndrome (MBS) falls within the CCDD category and at present the presumed genetic features remain unknown. MBS is most commonly defined as congenital, nonprogressive facial palsy, and abduction deficit, which is often considered to be the minimum diagnostic criteria (MDC). Unfortunately the literature still includes variable diagnostic criteria. A part of an on-going genetics of strabismus study the aim of this project was to improve the clinical diagnostic criteria for MBS.

**Materials & Methods:** Careful evaluation of ocular motility, ocular alignment, facial muscle function, as well as assessment of additional anomalies often reported with MBS were conducted in a large group of individuals previously given a diagnosis of MBS by their local health care providers. Research participants were recruited from 3 consecutive international Moebius Syndrome Conferences organized by the Moebius Syndrome Foundation.

**Results:** 112 participants were enrolled. Nineteen percent of participants (21/112) did not meet MDC for Moebius syndrome. Seven patients within this group were later confirmed to have one of three genetically distinct syndromes.

**Conclusions:** Moebius syndrome can easily be misdiagnosed due to variable diagnostic criteria used by health care professionals. Assessing individuals for presence of MDC improves accuracy of making the correct diagnosis. This project also supports the importance of accurate phenotyping particularly when research subjects are being selected for genetic testing. Recognition of MDC outliers led to the identification of three genetically distinct conditions that may have otherwise still been referred to as MBS. Conversely, having a genetic diagnosis helps confirm a diagnosis however the negative genetic results in these MBS patients further supports this as a distinct entity from other overlapping conditions

**15:33 RETURN OF RESULTS FOR IMPUTED MYOCILIN MUTATIONS IN GWAS STUDIES.** DAVID A MACKEY, PUYA GHARAHKHANI, KATHRYN P BURDON, ALEX W HEWITT, MATTHEW H LAW.  
*Perth, Australia*

**Introduction:** Myocilin has been studied for 18 years with the potential to feed back to patients and families the results of DNA testing. In most cases this has been received favourably and is useful in targeting clinical screening. To date Myocilin testing of unaffected individuals has not extended beyond cascade genetic screening of families, however the advent of whole exome sequencing will mean a large number of individuals with pathogenic Myocilin mutations may be identified incidentally. As this scenario has not arisen frequently to date and there are many other more serious diseases, this issue has not been discussed.

**Materials & Methods:** The most common Myocilin mutation, Gln368Ter, has an allele frequency of 0.001-0.003 in populations of European ancestry. Using Illumina Omni1M or OmniExpress arrays we could reliably impute the Gln368Ter variant (rs74315329), with a sensitivity of 100%, specificity of 99.91%, positive predictive value of 95.65%, and negative predictive value of 100% between imputation and sequencing.

**Results:** Among 3,793 individuals without POAG in this study, six were predicted (probability>95%) to carry the risk variant. We do not know the disease status in these individuals.

**Conclusions:** The American College of Medical Genetics and Genomics have been proactive in recommending return of incidental findings in clinical exome and genome sequencing. The conditions for which they recommend return of results include: Retinoblastoma, Tuberous Sclerosis, Von Hippel-Lindau syndrome, Familial adenomatous polyposis, and Marfan syndrome; all of which warrant an ophthalmological assessment. Should Myocilin glaucoma be another disease where incidental return of results is recommended? Should we inform the large number of people who already have data from arrays? Data on the penetrance of Gln368Ter in population screening is required to allow appropriate use of this information.

**15:45**      **Break/Poster Viewing**

**16:15**      **Symposium Ethics and Consenting**  
Moderator: Johane Robitaille

**16:15 ETHICAL CONSIDERATIONS OF INNOVATIVE THERAPY IN CHILDREN WITH UNILATERAL RETINOBLASTOMA.** CHRYSSA MCALISTER, BRENDA L GALLIE. *Toronto, Ontario Canada*

**Introduction:** To enhance the opportunity for families of children with unilateral retinoblastoma to make informed decisions about innovative treatment options, such as intra-arterial chemotherapy (IAC), and to guide physicians in developing care plans for these patients through an application of relevant ethical principles.

**Materials & Methods:** Comprehensive Medline database search to retrieve articles related to surgical innovation and ethics in ophthalmology and other surgical specialties. Relevant ethical values, principles, and frameworks, will be applied to the critical analysis.

**Results:** Innovative surgical treatments in children with retinoblastoma, such as IAC, attempt to provide alternatives to enucleation, the current standard of care.

However, they present challenges relating to safety and efficacy testing, informed consent, conflicts of interest, and beneficence. In contrast to the highly regulated environment of a clinical trial to test the safety and efficacy of an experimental drug or device, the process for evaluating surgical innovation is unregulated. This shortcoming is partially mitigated in frameworks and guidelines on the ethical development of innovative procedures: they recommend a rapid transition from case series to well-designed clinical trials to better protect patients from unknown risks. Despite this, no substantial clinical trials on the use of IAC in retinoblastoma have been published in peer-review journals or registered with the United States NIH. Informed consent for innovative treatments is challenged by a lack of information and understanding of potential risks, and a common public belief that newer options are inherently better. Consent is complicated by the vulnerabilities unique to families of retinoblastoma patients, facing possible eye removal, blindness and death. Conflicts of interest extend beyond financial incentives in the development of devices and include prestige in pioneering a field, a desire to attract referrals, and optimism bias towards the innovative treatment.

**Conclusions:** A search for eye-salvaging alternatives to primary enucleation in children with unilateral retinoblastoma must be weighed against their potential risk to life. A detailed informed consent process should extensively review benefits and harms of all treatment options. Further, clinicians must disclose all conflicts of interest, including those unique to pioneering treatments, and explain the innovative nature of relevant procedures. Innovative treatments like IAC require rigorous evaluation in collaborative well-designed clinical trials. The responsible surgeon should uphold their duty to “first, do no harm” when pioneering untested innovative treatments in unilateral retinoblastoma, a potentially lethal malignancy frequently cured by enucleation.

#### **16:45 PLANNING TO OFFER TARGET AND INCIDENTAL GENOMIC RESULTS. CONRAD**

FERNANDEZ *Halifax Nova Scotia, Canada*

**Introduction:** The power of genomic technology allows us to interrogate the human genome as never before. It has become an extremely important clinical and research tool. The extensive data that is generated challenges us as to how and whether or not we should offer individual genetic results to clinical and research participants. This discussion will review the current controversies and recommendations that arise in disclosing genomic results.

#### **17:15 OCULAR GENETICS: WHO SHOULD DO IT? ALEX LEVIN *Philadelphia, USA***

**Introduction:** As genetic testing becomes more accessible to physicians, the opportunity exists for more widespread use of this diagnostic tool. Yet, interpretation of results is a challenge and misinterpretation may lead to incorrect counseling. The paucity of ocular geneticists and the robust training programs for ocular genetics, simultaneously creates a resource challenge to provide care for patients with ocular genetic disease. Rarity of the disorders makes diagnostic familiarity difficult for physicians who do not routinely practice in the field. Balancing the ethical challenges of resource allocation, scope of practice, nonmaleficence and the desire to deliver robust care to the patients creates difficult issues which require a thoughtful response.

**18:00**

**Bus pick up from Conference Venue to Pier 21**

**18:30-21:00**

**Opening Reception - Pier 21**



## Friday August 7<sup>th</sup>

### 7:30 Ophthalmic Genetics Session 3 (Free papers)

Moderator: Godfrey Heathecote

#### **7:30 MYSTERY CASE.** ANN HOSKIN-MOTT. *Halifax, Nova Scotia, Canada*

**Introduction:** A 60 year-old mother and her 30 year-old son are presented with an unknown retinal dystrophy.

**Materials & Methods:** History, photos, and electrodiagnostics will be given with a 5 year follow-up. The mother began losing vision in her 30's and is now Hand Motions in each eye. The son's vision is 20/60 and 20/40

**Results:** The retinal dystrophy is not a recognized pattern, despite ng many specialists

**Conclusions:** Hope to generate discussion among the delegates and perhaps some diagnostic thoughts.

#### **7:42 A NOVEL MUTATION IN PRPH2/RDS GENE AND ITS SPECTRUM OF PHENOTYPIC MANIFESTATIONS IN A SYRIAN FAMILY WITH AUTOSOMAL DOMINANT RETINITIS PIGMENTOSA (ADRP).**

SUSAN WAKIL, RAZEK GEORGES COUSSA, CHRISTINA CHAKAROVA, VINCENT SUN, AYESHA KHAN, IRMA LOPEZ, HUANAN REN, KUNKA KAMENAROVA, SHOMI S. BHATTACHARYA, ROBERT K. KOENEKOOP. *Quebec, Canada*

**Introduction:** Retinitis pigmentosa (RP) is a clinically and genetically heterogeneous retinal disease that leads to blindness. Currently, about 50 genes explain ~50% of cases. We studied a Canadian family of Syrian origin with adRP and aimed to identify the causal gene.

**Materials & Methods:** Sanger sequencing allowed to exclude all known adRP genes. Next generation sequencing and whole exome capture were sequentially performed on selected patients at Otogenetics Corporation (Georgia, USA) and AROS Applied Biotechnology A/S (Aarhus N, Denmark). Phenotypes were characterized using visual acuity (VA), Goldmann visual fields (GVF), slit- lamp biomicroscopy, funduscopy, ERG (Diagnosys LLC), fundus autofluorescence (FAF), and Optical Coherence Tomography (OCT) (Heidelberg Engineering Inc, Heidelberg, Germany).

**Results:** We identified a novel heterozygous mutation in PRPH2/RDS (del352\_356TGCTGinsCT) in all affected individuals. The initial proband (MOGL 322) showed severe progressive disease with non-recordable ERGs as well as typical GVF tunnel constriction. The other patients showed a wide spectrum of phenotypic manifestations ranging from normal fundi and FAF to severely and abnormal ERGs.

**Conclusions:** We have successfully identified a novel mutation in PRPH2/RDS leading to adRP. We are currently investigating the full extent of the mutation spectrum and severity. Our findings are crucial in expanding the current understanding of inherited retinal blinding diseases in order to provide new avenues for therapeutic interventions.

#### **7:54 A CASE OF JALILI SYNDROME.** MARK PENNESI, AKSHAY THOMAS, ZHONGQI GE, RUI CHE. *Portland, Oregon.*

**Introduction:** Jalili syndrome is a rare genetic disease caused by mutations in the CNNM4 gene and characterized by a cone-rod dystrophy and enamel hypoplasia (amelogenesis imperfecta).

**Materials & Methods:** A 6 year-old Caucasian male presented OHSU Casey Eye Institute for evaluation of decreased vision and nystagmus. since the age of 6 months. He had a history of enamel hypoplasia.

Family history revealed that the proband's mother had a cousin (age 32) with retinitis pigmentosa and another cousin with enamel hypoplasia but normal vision (age 22). Routine imaging and electrophysiological testing was performed

**Results:** Best corrected visual acuity was 20/250 OD and 20/200 OS. Anterior segment examination was unremarkable. Fundus examination showed tilting of the optic discs, loss of the foveal light reflex and mild vascular attenuation OU. Kinetic visual fields showed normal responses to the larger peripheral isopters but constriction to the smaller isopters.

Multifocal ERG revealed unrecordable macular cone responses OU. Full-field ERG demonstrated mostly normal rod-driven recordings but severely decreased cone driven responses OU. SD-OCT demonstrated severe outer retinal atrophy OU. Fundus autofluorescence showed generalized hyper-autofluorescence in the posterior pole as well as a ring of hyper-autofluorescence around the fovea.

**Conclusions:** Genetic testing revealed two heterozygous mutations in CNNM4. The first mutation was c.1307delC, p.T436fs, a novel frameshift predicted to be deleterious. The second mutation was c.C1690T, p.Q564X, a known stop-gain. These CNNM4 mutations confirmed the diagnosis of Jalili syndrome.

#### **8:06 PHENOTYPIC VARIATION IN AFFECTED MEMBERS OF A FAMILY HARBORING AN NDP GENE MUTATION.** IRINA DE LA HUERTA, JACQUE L. DUNCAN, ANNE SLAVOTINEK, ANTHONY T. MOORE, ALEJANDRA G. DE ALBA CAMPOMANES. *San Francisco, California*

**Introduction:** This work can be presented either as a poster or as a paper for podium presentation. Norrie disease is an X-linked recessive disorder characterized by retinal vascular dysplasia that typically presents with blindness soon after birth. X-linked familial exudative vitreoretinopathy is a disorder characterized by incomplete peripheral retinal vascularization. Both diseases are caused by mutations in the NDP gene which encodes the protein Norrin. Over 75 disease-causing mutations in the NDP gene have been identified; however the phenotypic variability associated with each mutation has not been well-characterized. Here we describe a spectrum of phenotypes in three generations of a family harboring a disease-causing mutation in the NDP gene.

**Materials & Methods:** Testing for mutations in the NDP gene and in the genes associated with autosomal dominant forms of FEVR, Fzd4 and LRP5, was performed on genomic DNA isolated from peripheral venous blood. Full ophthalmic examinations were obtained. Fluorescein angiography and ultrasound biomicroscopy were performed where applicable.

**Results:** No mutations were found in the Fzd4 and LRP5 genes. A single nucleotide substitution, c.529C>T (p.Arg38Cys), in the NDP gene was identified in the male proband, in his brother, his mother, and his maternal grandfather. The NDP mutation caused a severe phenotype in the proband with bilateral total retinal detachments by age 2. His brother was found at age two months to have incomplete vascularization of the peripheral retina with evidence of neovascularization and preretinal hemorrhages. The proband's maternal grandfather, affected

by the same NDP gene mutation, was clinically asymptomatic but with avascular peripheral retina and fibrotic neovascular tufts bilaterally. His daughter (the proband's mother), a carrier of the c.529C>T NDP mutation, was also clinically asymptomatic, but was revealed on examination and fluorescein angiography to have avascular far peripheral retina in the right eye.

**Conclusions:** The c.529C>T NDP mutation may be responsible for different phenotypes in affected males from the same family. This mutation is associated with retinal vascular abnormalities in a female carrier.

### **8:18 RECOGNIZING LYONIZATION IN FEMALE HETEROZYGOTES OF X-LINKED OCULAR DISEASE IN THE ERA OF MOLECULAR DIAGNOSTICS. BART P LEROY, ELKE O KREPS, ELFRIDE DE BAERE, JULIE DE ZAEYTIJD. GHENT, BELGIUM**

**Introduction:** To investigate the clinical accuracy of recognizing female heterozygotes for X-linked retinitis pigmentosa (XLRP) and choroideraemia (CHM), using fundoscopy and blue-light fundus autofluorescence (FAF).

**Materials & Methods:** Retrospective analysis of data on 24 female XLRP heterozygotes from 15 different families (age 3-77 years), and 8 CHM heterozygotes from 5 families (age 14-65 years). Molecular diagnosis has been obtained for all subjects.

**Results:** For XLRP, 16 of 24 heterozygotes (66.7%) mentioned decreased night vision. RPGR mutations were identified in 17 subjects - 8 of which in ORF15 - whereas a causative mutation was found in RP2 in the remaining 7 heterozygotes. Dilated fundus examination showed no abnormalities in 8 subjects, a tapetoid reflex in 2, regional pigmentary changes with or without bone spiculae in 15 and full-blown RP features in 1 heterozygotes. Both subjects with a tapetoid reflex - aged 18 and 34 - had a mutation in RPGR-ORF15. An abnormal FAF pattern was found in 16 of 24 subjects (70.8%). Of the

24 molecularly proven female XLRP heterozygotes, 21 (87.5%) showed abnormalities on fundoscopy and/or FAF. In CHM, only 1 of 8 subjects - aged 40 - mentioned visual difficulties at night. In each of the 8 heterozygotes, typical, equatorial, mottled pigmentary changes were evident. FAF revealed multiple small hyper- and hypoautofluorescent flecks in all 8 subjects. In both XLRP and CHM, clinical findings were independent of age or specific mutation.

**Conclusions:** Female heterozygotes of X-linked retinitis pigmentosa show abnormalities on dilated fundoscopy and/or blue-light fundus autofluorescence in 87.5% of cases with a molecularly proven diagnosis. The most characteristic feature is a radial pattern of alternating areas of hyper- and hypoautofluorescence. In choroideremia, all carriers exhibit pigmentary changes in the retinal midperiphery and scattered autofluorescence changes, despite a lack of visual symptoms. Thus, fundoscopy and blue-light AF imaging are sensitive means of detecting female heterozygotes in choroideremia.

**8:30**      **François Lecture: Richard Weleber, Portland, USA**  
Clinical Trials for Inherited Retinal Diseases: New Endpoints, Analyses for Efficacy, and Model for Progression  
Introduction by Bart Leroy

**9:15**      **Break/Poster Viewing**

**9:45**      **Symposium on Congenital Cataracts**  
Moderator: Irene Maumenee

**9:45 PHENOTYPE-GENOTYPE CORRELATIONS IN PEDIATRIC CATARACT.** ARIF O KHAN, *Riyadh, Saudi Arabia*

**Introduction:** This presentation highlights phenotype-genotype correlations for pediatric cataract.

**Materials & Methods:** Didactic lecture, illustrative case presentations.

**Results:** Certain specific diagnoses or gene mutations can be suggested by pediatric cataract morphology and/or accompanying dysmorphic/systemic features of the child.

**Conclusions:** Although pediatric cataract phenotypes are generally non-specific, there exist notable exceptions that should be kept in mind.

**10:15 MOLECULAR TESTING FOR CONGENITAL CATARACT.** GRAEME CM BLACK, RACHEL GILLESPIE, IAN CHRISTOPHER LLOYD, JILL CLAYTON-SMITH, JANE ASHWORTH. *Manchester, United Kingdom*

**Introduction:** To define the efficacy of high throughput genomic testing in the diagnosis of bilateral congenital cataract

**Materials & Methods:** 100 individuals diagnosed with isolated non-syndromic or syndromic bilateral congenital cataract were selected for investigation through a single ophthalmic genetics clinic. Participants underwent a detailed ophthalmic examination, accompanied by dysmorphology assessment where appropriate. Mutation detection was performed using a custom designed target enrichment which permitted parallel analysis of 115 genes associated with CC by high throughput next generation DNA sequencing (NGS). Suspected pathogenic variants were confirmed by bidirectional Sanger sequencing in relevant probands and other affected family members.

**Results:** NGS technologies are able to determine the precise genetic cause of CC in 75% of individuals. Around 40% of variants are missense alterations which represents a significant challenge for clinical interpretation. Individuals with unsuspected syndromic diagnoses, as well as those with potentially treatable underlying causes will be used to demonstrate the clinical utility of testing.

**Conclusions:** Implementation of genomic testing early in the care pathway for children with CC can alter clinical management, direct care pathways and enable accurate genetic counselling.

This leads to improved diagnostic and management outcomes through a stratified medicine approach.

**10:45 GENETIC TESTING FOR CONGENITAL CATARACTS.** ARLENE DRACK, EDWIN STONE, SCOTT LAMBERT, SUSANNAH LONGMUIR, SCOTT LARSON, RICHARD OLSON, ADAM DELUCA, JEREMY HOFFMAN, MATTHEW WEED. *Iowa City, Iowa*

**Introduction:** Congenital cataracts are a major cause of treatable blindness in children worldwide. The large number of causative genes and the autosomal dominant, recessive, and X-linked recessive inheritance patterns make genetic testing complex. We present a strategy for genetic testing of congenital cataracts and discuss the features of this disorder that make genetic diagnosis especially challenging.

**Materials & Methods:** IRB approval was obtained. Congenital cataract patients presenting to two pediatric ophthalmology services were offered research-based genetic testing. Literature review of causative mutations published at least twice was used to develop a Sanger sequencing pre-screen of 24 exons in 11 genes (BFSP2, CRYAA, CRYBA1, BRYBB2, CRYGD, EPHA2, FAM126A, FYOC1, NHS, PAX6 and VSX2). Negative pre-screens received exome sequencing on a lens-related genes panel. Variants were verified by Sanger sequencing. Allele frequency, calculated effect of mutations, and familial segregation were used to impute pathogenicity.

**Results:** 31 proband samples have been completely analyzed. Pre-screening identified definite causative variants in 3 families and probable in another 2 (5/31 or 16%). Twenty six of the 31 probands had negative pre-screen and went on to exome sequencing. 23/26 had protein-altering mutations in one or more reported congenital cataract genes (88%). One of these mutations did not segregate with the cataract in the family. Disease-causing genes identified on pre-screen included CRYBA1(1), CRYGD (2) and NHS (1). Probably disease-causing variants are in CRYAA and FYCO1. In several probands a very plausible disease-causing mutation was found that was later disproven after further analysis of other family members, copy number variant testing, or other means.

**Discussion:** Exome sequencing can be used to simultaneously screen multiple genes for disease-causing mutations and is useful for a polygenic disease like congenital cataract. Non-disease causing variants are common in humans, however; results must be carefully scrutinized to confirm pathogenicity. A pre-screen test for known, common mutations decreases cost and increases testing efficiency.

**Conclusion:** Congenital cataract genetic testing combining a pre-screen with an exome platform allows clinicians to provide genetic counseling, prognosis, and early differentiation of syndromic from non-syndromic cataract.

**11:15 Ophthalmic Genetics Session 4 (Free papers)**  
Moderator: Birgit Lorenz

**11:15 A UNIQUE CASE OF SJOGREN RETICULAR DYSTROPHY AND NEW FINDINGS ON MULTIMODAL IMAGING.** R. RISHI GUPTA, NETAN CHOUDHRY, ANN HOSKIN-MOTT. *Halifax, Nova Scotia, Canada*

**Introduction:** We would like to give a podium presentation (the images and flow of presentation are more conducive to podium rather than poster.)

**Purpose:** To describe the phenotype in a unique case of Sjogren reticular dystrophy.

**Materials & Methods:** We present multimodal imaging, including ultra-widefield (UWF) pseudocolour, autofluorescence, and fluorescein angiography images. In addition, we present the first descriptions of Enhanced-depth imaging optical coherence tomography (EDI-OCT) of the choroid in this disease, as well as en-face SD-OCT images. Visual field testing, formal colour vision testing (Farnsworth- D15), and electroretinogram (ERG) studies were performed.

**Results:** Colour photos demonstrated a classic "reticular" pigmentary pattern in the posterior pole that was symmetrical between the two eyes. Autofluorescence imaging showed a mixture of hypo- and hyper-autofluorescence. In each eye, there was significant atrophy in the temporal peripapillary area that showed hypo- autofluorescence, and stained intensely on fluorescein angiography. The choroid when assessed with EDI-OCT appeared normal. En-face OCT demonstrated hyporeflectivity in areas that corresponded to atrophy on colour photographs. The reticular pigment observed on colour photographs appeared hyperreflective on en-face OCT. The ERG however, revealed approximately a 50% reduction in rod and cone mediated responses.

**Conclusions:** We describe new imaging with EDI-OCT and en-face OCT in a patient with Sjogren reticular dystrophy, a very rare, autosomal recessively inherited disease. We present reduced ERG findings in the disease " only the second report of this in a condition that otherwise has been described to have normal ERG.

**11:27 CASE OF MICROPTHALMIA, ANIRIDIA, PERSISTENCE OF HYALOID VASCULATURE, AND RETINAL DYSTROPHY.** YUSUKE MATSUKANE, HIROYUKI KONDO. *Fukuoka, Japan.*

**Introduction:** Microphthalmia is a heterogeneous disorder characterized by a variety of ocular and systemic anomalies. Microphthalmia can be caused by mutations in a single or multiple genes and also by systemic infections or alcohol abuse during pregnancy. We report a sporadic case of microphthalmia with a unique association of, aniridia, persistent fetal vasculature (PFV), and retinal dystrophy which was negative for a PAX6 mutation.

**Materials & Methods:** A 2-month-old girl was born with normal pregnancy and delivery. She was noted to have esotropia and microphthalmia at birth. She had no family history of ocular anomalies. Ocular examinations were performed including measurements of the visual acuity, slit-lamp examination of the anterior and posterior segments, ophthalmoscopy, ultrasonography, and electroretinography (ERG). Sanger sequencing of the exons of the PAX6 gene, and whole exome sequencing were performed on her DNA.

**Results:** Her visual acuity was followed from 6 month of age. Ocular examinations revealed narrowed eyelids of 11 mm with inner canthus vegetation, microcornea of 6 mm, aniridia, and

retinal degeneration with hypoplasia of the optic disc. Her parents appeared not to have any ocular anomalies. B-mode ultrasonography showed a persistence of the hyaloid vasculature in both eyes, and the axial lengths were 17.7 mm OD and 16.9 mm OS. The dark-adapted a- and b-wave of the single flash ERGs were reduced, and the implicit times of the flicker ERGs were delayed. Mutation analyses were negative for the PAX6 gene. Whole exome sequencing showed no significant DNA changes of known microphthalmia-associated genes listed in OMIM, except for a heterozygous p.H251Y change in the BMP4 gene.

**Conclusions:** A case with an association of microphthalmia, aniridia, PFV, and retinal dystrophy has not been reported. The BMP4 gene is known to cause syndromic microphthalmia, but a p.H251Y alteration is probably not likely to be causative because of a lack of cosegregation and a population frequency of 0.5%. We conclude that this case was caused by mutation of genes other than the known microphthalmia-associated genes.

### **11:39 BROTHERS WITH CONGENITAL OCULAR MOTOR APRAXIA, JUVENILE**

**NEPHRONOPHTHISIS AND MILD CEREBELLAR DEFECTS.** STEPHANIE CHAN, JASPREET RAYAT, YVES SAUVÃ, IAN M. MACDONALD. *Alberta, Canada*

**Introduction:** Congenital ocular motor apraxia (COMA) is a disorder of absent or flawed horizontal eye movements. Head thrusts are used to compensate for poor saccades. A small subset have extraocular findings including Joubert syndrome or juvenile nephronophthisis. We report brothers with a large homozygous NPHP1 deletion who presented with COMA, panretinal dysfunction, nephronophthisis and mild cerebellar defects.

**Materials & Methods:** Renal failure was first indicated by ultrasound and elevated plasma creatinine levels. Nephronophthisis was identified by histology. NPHP1 genetic testing by linkage analysis, full field electroretinograms(ERGs) and magnetic resonance imaging (MRI) of the brain in adulthood were performed on both brothers.

**Results:** A non-functioning multicystic kidney was detected by renal ultrasound in the younger brother and subsequently removed at one year of age. Then at age 9, this brother presenting with weight loss and lethargy, was diagnosed with mild renal failure. The older brother had elevated plasma creatinine at age 11 and within a year he presented with renal failure. Nephronophthisis was confirmed by histology in both brothers. The siblings have both since had renal transplants. Linkage analysis revealed that both brothers carried a large homozygous NPHP1 deletion, confirming the diagnosis of juvenile nephronophthisis. According to International Society for the Clinical Electrophysiology of Vision standards, full field ERGs from the two brothers (age 11 and 13) showed panretinal dysfunction affecting cone-driven responses. Photopic b-wave amplitude reductions were more pronounced in the younger brother. The younger brother showed amplitude reductions in the pure rod b-wave as well as in both the mixed a- and b-waves, while scotopic responses were normal in the older brother. Recordings two years later confirmed these observations. Brain MRIs in adulthood showed that both siblings had the molar tooth deformity (cerebellar vermis hypoplasia and thinned superior cerebellar peduncles) in keeping with Joubert syndrome; the deformity was greater in the younger brother.

**Conclusions:** Observations in these brothers further support evidence that COMA may present with extra-ocular manifestations (nephronophthisis and cerebellar vermis hypoplasia), and that COMA can be associated with NPHP1 mutations. Our ERG results also provide further evidence

for retinal involvement in COMA. Our findings support offering renal screening for patients with COMA and confirmed NPHP1 mutations even if the patient is too young to present with renal dysfunction. In addition, neuroimaging can determine whether a diagnosis of Joubert syndrome should be considered.

**11:51 NOVEL HPS6 GENE MUTATIONS IDENTIFIED BY WHOLE-EXOME SEQUENCING IN JAPANESE TWO SISTERS WITH OCULAR ALBINISM.** DAISUKE MIYAMICHI, MIKI ASAHINA, JUNYA NAKAJIMA, MIHO SATO, KATSUHIRO HOSONO. *Shizuoka-ken, Japan*

**Introduction:** To report two sisters with ocular albinism (OA) caused by HPS6 mutations. They were analyzed all exons and surrounding area of the 5 causative genes (TYR, OCA2, TYRP1, SLC45A2, and GPR143) for OA and oculocutaneous albinism (OCA) by Sanger sequencing, and no mutation was recognized. In this time, whole-exome sequencing (WES) identifies gene mutations responsible for Hermansky-Pudlak syndrome (HPS) in two sisters.

**Materials & Methods:** A 3-year-and-11-month-old girl was referred to us with poor vision. She showed congenital nystagmus, exotropia and iris translucency. Her hair color was light brown and skin color was fair. The ocular fundus was albinotic with bilateral foveal hypoplasia. We examined her younger sister at 5 month-old. She presented nystagmus with impaired vision. Her fundus was albinotic with bilateral foveal hypoplasia. Her hair color was also light brown and skin color was fair. Their hair and skin color seem to be within normal limit for Japanese people. They got suntans in summer. They showed only ocular findings without general findings including bleeding problems in follow-up period of 56months. Clinical features strongly suggested the OA in two sisters.

In this study, to identify the causative mutations, we performed WES in elder sister and her parents. Written informed consent was obtained from the parents before molecular genetic studies were performed. Genomic DNA was extracted from peripheral lymphocytes of the two sisters and their parents by using standard procedures. Two candidate genes were extracted from data obtained by WES. The identified possible pathogenic mutations were confirmed using Sanger sequencing.

**Results:** A novel compound heterozygous mutations of HPS6 (c.1897delC:mother origin and c.2038C>T:father origin) were identified in two sisters. HPS6 is one of the causative genes of Hermansky-Pudlak syndrome (HPS) which is complicated with OCA. WES is a comprehensive method that has enabled the analysis of large amounts of genes at efficient, time-saving, and reasonable costs. Although they showed only ocular findings without general findings including bleeding problems, WES identifies atypical cases of HPS.

**Conclusions:** The sequencing throughput has dramatically increased and the costs have decreased with WES. WES is beneficial to diagnosis of an unusual HPS phenotype.

**12:05**      **Matthew Herder (Canada)**

Orphan drug policies: Lessons learned?

**12:30**      **ISGEDR Business Meeting**



**13:00**      **Lunch/Poster Viewing**

**14:00**      **Symposium Retinoblastoma**  
**Imaging in RB - Driving Clinical Innovations**  
Moderator: Brenda Gallie

**14:00 Francis Munier (Switzerland)**

**14:25 Junyang Zhao (China)**

EUA and the retina camera application on retinoblastoma diagnosis

**14:50 Cynthia Vandenhoven (Canada)**

**15:15 MULTIMODALITY IMAGING OF RETINOBLASTOMA ANIMAL MODELS.** TIMOTHY CORSON. *Indianapolis, IN*

**Introduction:** Animal models are a mainstay of preclinical research in retinoblastoma. In particular, orthotopic xenografts and transgenic rodent models are popular. As in the human disease, non-invasive imaging by methods such as optical coherence tomography (OCT) can provide crucial information about tumor size, location, and development over time.

**Materials & Methods:** Newborn rats orthotopically xenografted with fluorescent and bioluminescent retinoblastoma cell lines were studied by brightfield and fluorescence funduscopy, bioluminescence imaging (BLI), and OCT. The TAg-RB transgenic mouse model of retinoblastoma was studied over time by funduscopy and OCT.

**Results:** BLI of orthotopic xenografts allowed quantification of tumor burden, while brightfield funduscopy revealed tumor morphology and location, confirmed by fluorescence funduscopy. OCT provided depth and localization information unobtainable with other methods, plus the ability to rapidly screen for xenograft formation. In TAg-RB mice, OCT enabled detection of the earliest intraretinal tumor cell clusters before they were evident by funduscopy, as well as longitudinal monitoring of tumor formation, including differences induced by genetic manipulation. Tumor volume could be estimated based on OCT analysis.

**Conclusions:** Advances in preclinical imaging permit unprecedented qualitative and quantitative analyses of retinoblastoma animal models. These methodologies will allow characterization of new models, longitudinal studies of genetic factors, and therapeutic trials in future.

**14:00**      **Ophthalmic Genetic Counselor's Breakout Session**  
Moderator: Meghan Marino

**14:00**      **Introduction.** Meghan Marino

**14:02**      **Ophthalmic GC Collaborations and Community Outreach.** Karmen Truzpek

**14:22**      **Billing Challenges, Barriers to Services, And Solutions.** Dianna Wheaton And Kari Branham

**14:42**      **Counseling/Testing Standards & NSGC Practice Guidelines.** Meghan Marino

**15:02**      **Ethical Dilemmas.** Jill Beis

**15:17**      **Closing Remarks And Future Plans.** Meghan Marino

**16:00 - 19:00**

**Trip to Peggy's Cove**

Bus pick up at 16:00 from Conference Venue  
and 19:00 from Peggy's Cove (return 20:00 in Halifax)

**FREE EVENING IN HALIFAX**

**Saturday August 8<sup>th</sup>**

**8:00 Symposium: Developmental Genes and Eye Malformations**

*Symposium in memory of Mette Warburg*

Moderator: Elias I. Traboulsi



**8:00 METTE WARBURG.** ELIAS I. TRABOULSI. *Cleveland, Ohio*

**8:10 ADAMTS PROTEINS, IMPORTANT PARTICIPANTS IN OCULAR MORPHOGENESIS AND GENETIC DISORDERS.** SUNEEL APTE. *Cleveland, Ohio*

**Introduction:** The connective tissue and extracellular matrix (ECM) of the eye provides the underpinning for several structures and is indispensable for some of them such as the zonule, comprising fibrillin microfibrils, and the lens capsule, comprising basement membrane molecules. ADAMTS proteases are a large family of secreted metalloproteases implicated in connective tissue assembly and turnover. ADAMTS-like proteins resemble them, but lack a protease domain and are not proteases, but secreted ECM proteins. Together, these ADAMTS proteins have emerged as significant molecules in the eye that are implicated by the genetics of Mendelian and non-Mendelian disorders.

**Materials & Methods:** The genetics of human and canine Mendelian conditions that affect the ocular zonule (resulting in ectopia lentis or ectopia lentis et pupillae) has identified anomalies resulting from mutations in ADAMTS proteases and ADAMTS-like proteins, specifically, ADAMTS10, ADAMTS17 and ADAMTSL4. This work was further advanced by reverse genetics in mice, which also resulted in identification of new roles for ADAMTS proteins in eye development and disease. In situ hybridization has been used to define the expression patterns of these ADAMTS genes in mice and humans. Recombinant ADAMTS proteins were used to understand their interactions with fibrillin microfibrils.

**Results:** Through analysis of null mouse mutants and recombinant proteins, we have found that ADAMTS10, ADAMTSL4 and ADAMTS17 interact with fibrillin microfibrils and affect their assembly. The expression pattern of ADAMTS10, ADAMTS17 and ADAMTSL4 indicated specific expression in the ciliary body or lens epithelium, the two sites of zonule assembly. ADAMTS9

haploinsufficiency in mice leads to anterior segment dysgenesis with Peters Anomaly, a small lens and posterior and anterior lens capsule defects. Adamts9 is expressed in both the anterior and posterior eye segments, and a non-autonomous role is suggested by conditional deletion in the optic cup using alpha-Cre, which also led to ASD. We have found that ADAMTS9 is a substrate for modification by the glucosyltransferase B3GLCT, which is required for its secretion. In humans, B3GLCT mutations lead to a recessive condition called Peters Plus Syndrome.

**Conclusions:** Zonule assembly, its stability and/or maintenance clearly require ADAMTS proteins, which likely also affect other parts of the eye and extraocular tissues. The presence of Peters anomaly in Adamts9 haploinsufficient mice and its reduced secretion by suppression of B3GLCT, suggests that ADAMTS9 impairment is a major underlying mechanism in PPS.

**8:32 CONSERVED GENETIC PATHWAYS IN MICROPTHALMIA, ANOPHTHALMIA AND COLOBOMA.** ELENA V. SEMINA\*, BRETT DEML, ARIANA KARIMINEJAD, RAZIEH H. R. BORUJERDI, SANAA MUHEISEN, LINDA M. REIS. *Milwaukee, USA*

The human eye formation requires extraordinary coordination of various developmental processes. The conservation of developmental steps in vertebrates suggests possible common genetic mechanisms. Genetic diseases involving the eye represent a leading cause of blindness in children and adults. During the last decades, there has been an exponential increase in genetic studies of ocular disorders. Using whole exome sequencing we recently identified a novel heterozygous allele in *MAB21L2*, c.151 C>G, p.(Arg51Gly) in a family affected with dominant coloboma, microcornea, cataracts, and skeletal dysplasia. *MAB21L2* encodes a protein of unknown function and similar to *C. elegans* mab-21 cell fate-determining factor. To evaluate the identified variant, zebrafish mutants carrying a p.(Gln48Serfs\*5) frameshift truncation and a p.(Arg51\_Phe52del) in-frame deletion were developed with TALEN technology. Homozygous zebrafish embryos from both lines demonstrated variable lens and coloboma phenotypes. Protein studies showed decreased stability for the human p.(Arg51Gly) and zebrafish p.(Arg51\_Phe52del) mutant proteins and predicted a complete loss-of-function for the zebrafish p.(Gln48Serfs\*5) frameshift truncation. Functional conservation between human *MAB21L2* and zebrafish *mab21l2* factors allowed for evaluation of human alleles in zebrafish model. In contrast to wild-type human *MAB21L2* transcript, mutant p.(Arg51Gly) mRNA failed to efficiently rescue the ocular phenotype of homozygous p.(Gln48Serfs\*5) embryos, suggesting this allele is functionally deficient. These findings support the identification of *MAB21L2* as a novel factor involved in human coloboma and further highlight the conservation of genetic pathways involved in ocular vertebrate development. The animal models allow for further investigation of the mechanisms of ocular phenotypes, integration of various identified genes into common developmental pathways and finally, provide an avenue for the development and testing of therapeutic interventions.

**8:54 RECESSIVE MICROPTHALMIA SYNDROMES.** NICOLA RAGGE. *Oxford, UK*

Developmental eye anomalies, including anophthalmia (absent eye), microphthalmia (small eye) (A/M) and coloboma are a heterogeneous collection of disorders with an overall frequency of around 1-3/10,000 live births. Over 50% of individuals affected by A/M have additional systemic features, including developmental delay, autism, pituitary anomalies, seizures,

deafness, and heart and kidney anomalies. Over 250 genes are now known to cause eye anomalies. This presentation will review the range of recessive microphthalmia syndromes that have been identified, including those relating to syndromes described by Mette Warburg, such as Walker-Warburg syndrome (FKRP). Other conditions include Matthew Wood or PDAC syndrome (STRA6), Waardenburg anophthalmia syndrome (FNBP4) and syndromes involving SMOC1, VSX2, FOXE3, ALDH1A3, RAX and RARB.

**9:16 THE TRANSCRIPTION FACTOR FOXC1 DIRECTLY REGULATES PROSTAGLANDIN RECEPTORS: A POTENTIAL EXPLANATION FOR RECALCITRANT GLAUCOMA IN AXENFELD-RIEGER SYNDROME.** MICHAEL WALTER, TIM FOOTZ, LANCE DOUCETTE. *Alberta, Edmonton, Canada*

**Introduction:** Individuals with mutations in FOXC1 have Axenfeld-Rieger syndrome (ARS), an ocular syndrome in which >50% of patients develop glaucoma. Individuals with FOXC1 mutations do not respond to prostaglandin glaucoma drugs, a common treatment for this condition. We hypothesize that a dysregulation of prostaglandin receptors (FP and EP1-4) by FOXC1 is the basis for this lack of response.

**Materials & Methods:** ChIP and luciferase assays were used to show that FOXC1 can bind to and regulate a fragment in the EP3 gene. To examine changes in RNA and protein levels of EP1,2,3,4 and FP in response to FOXC1, we used quantitative PCR (qPCR) and Western analysis respectively. QPCR analysis of MMP expression was used to elucidate the biological effect of lowering levels of FOXC1 on prostaglandin receptor responses.

**Results:** FOXC1 directly binds and regulates EP3 as revealed by ChIP and Luciferase assays. To examine if this translates to altered expression of the prostaglandin receptors, we conducted FOXC1 overexpression and siRNA knockdown studies. Our data shows that EP2 ( $p=0.0354$ ), EP3 ( $p=0.0004$ ), and EP4 ( $p=0.00005$ ) RNA expression is directly correlated to levels of FOXC1 while EP1 and FP seem to be unaffected. Preliminary evidence indicates that lowering FOXC1 levels changes MMP1 levels in response to the prostaglandin analogue Latanoprost.

**Conclusions:** FOXC1 can directly bind to and regulate the expression of the EP3 gene. QPCR and western data for the remaining EP receptors as well as FP suggest a positive correlation between levels of FOXC1 and EP2, EP3, and EP4. Preliminary results indicate that lowered FOXC1 levels, as would occur in patients with ARS mutations, results in an impaired response to the common glaucoma medication Latanoprost. This finding has downstream potential to change the course of treatment for ARS patients, and may provide a molecular explanation for the recalcitrant glaucoma suffered by many ARS patients.

**9:38 NEW POSTNATAL THERAPEUTIC OPTIONS FOR CONGENITAL OCULAR MALFORMATIONS.** CHERYL GREGORY-EVANS, XIA WANG, KISHOR WASAN, KEVIN GREGORY-EVANS. *Vancouver, BC*

**Introduction:** The extensive genetic and allelic heterogeneity observed with congenital eye diseases is a major barrier to the development of therapeutics. Leber congenital amaurosis is a typical example where more than 400 mutations have been identified in at least 20 different genes. Conversely, more than 600 mutations in a single gene (PAX6) cause aniridia. Developing treatments for each affected gene remains a significant challenge and would have to overcome the ethical and technical barriers associated with the delivery of a preventative prenatal treatment. To circumvent these issues we have taken advantage of the observation that

approximately 12% of all disease-causing mutations are nonsense mutations leading to premature stop codons. Our approach has therefore focused on pharmacological strategies that specifically target nonsense mutations and are gene-independent.

**Materials & Methods:** Mutant zebrafish embryos (rep1 and pax2) were dosed with the nonsense suppression drug gentamicin for 7 days and then the morphological phenotype compared to untreated controls. Transgenic rats (RhoS334X) or mice carrying nonsense mutations (Pax6 and Rpe65) were treated with 30ug/g daily subcutaneous injections of either gentamicin or Ataluren. In addition Pax6 mice also received topical START therapy containing Ataluren. Efficacy of treatment was assessed by histology, ELISA, electroretinography and optokinetic tracking behavior.

**Results:** We used systemic prenatal and postnatal nonsense suppression to correct the underlying histological defects in pre-clinical models of choroideremia, ocular coloboma, aniridia, retinitis pigmentosa and most recently Leber congenital amaurosis. During these studies we developed a topical eye drop formulation (called START therapy) containing the nonsense suppression drug Ataluren that we tested in a mouse model of aniridia. Topical delivery not only reversed the malformation defects it also restored the electrical and behavioral responses of the retina. Further studies on the efficacy of this postnatal treatment have shown that the eye responds to changes in Pax6 dosage over a specific time window and that the lens is particularly sensitive to Pax6 dosage.

**Conclusions:** These studies suggest that the eye retains significant developmental plasticity into the post-natal period and therefore post-natal therapeutic strategies delivered early in life could now be considered as potentially practical for some congenital eye abnormalities. Not only would topical drug delivery avoid the issue of systemic toxicity, but would likely lead to high compliance in young children. Furthermore, this approach would be relevant to new mutations that spontaneously occur without family history.

**10:00**      **Break/Poster Viewing**

**10:30**      **Ellsworth Lecture: Junyang Zhao (China)**  
Retinoblastoma in China: past, present and future  
Introduction by Brenda Gallie

**11:45**      **Ophthalmic Genetics Session 5 (Free papers)**  
Moderator: Bart Leroy

**11:45 ANTISENSE OLIGONUCLEOTIDE SELECTIVELY TARGETING THE P23H VARIANT OF RHODOPSIN FOR THE TREATMENT OF P23H RHODOPSIN-MEDIATED ADRP.** MICHAEL L. MCCAULEB, ALI JAZAYERI, RAECHEL PERALTA, SHULING GUO, BRETT P. MONIA, SUE F. MURRAY.  
*Carlsbad CA*

**Introduction:** The objective of the study was to identify an allele-specific antisense oligonucleotide (ASO) targeting the P23H variant of human rhodopsin mRNA (hRHO), the most common cause of ADRP in American patients. P23H RHO ASO will be selective for the P23H

allele, sparing normal (WT) mRNA expression, resulting in a reduction of the mutant protein and allowing WT protein expression and processing, and maintenance of rod survival and function.

**Materials & Methods:** Cell lines expressing human WT and P23H RHO sequences were used to identify potent and selective P23H RHO ASOs. Leads were evaluated in vivo using transgenic (Tg) mice expressing one allele of the P23H hRHO gene or Tg mice expressing one allele of the human WT RHO gene after administration of either a human P23H specific ASO or a control non-rhodopsin ASO by intravitreal injection (IVT). To assess therapeutic potential of the ASO, the lead P23H RHO ASO was evaluated for activity and selectivity in cynomolgus monkeys.

**Results:** ISIS 664844 had an in vitro IC50 of 2  $\mu$ M in P23H hRHO expressing cells and  $>40$   $\mu$ M in cells expressing WT hRHO. The ASO targeting the human P23H RHO sequence achieved a  $42\pm 5\%$  reduction in mutant RHO mRNA expression 7d following a 50  $\mu$ g IVT injection in the P23H RHO Tg mice. There was no reduction in the control non-rhodopsin ASO treated eyes ( $0\pm 3\%$ ). ISIS 664844, did not significantly reduce human WT RHO expression. Evaluation of 664844 in monkeys demonstrated no significant reduction in monkey WT RHO expression at 150  $\mu$ g ( $3 \pm 3\%$ ;  $p>0.05$ ) 10 wk after a single IVT injection as compared to PBS-injected eyes. As a positive control, a monkey active ASO targeted against the WT RHO sequence achieved a  $60\pm 7\%$  reduction after a single IVT injection at 400  $\mu$ g. No structural or functional changes were observed in the eyes treated with 664844, as determined by ophthalmological exam, histological exam or ERG analysis.

**Conclusions:** We have identified and characterized a human RHO P23H allele-specific ASO, ISIS 664844, which targets the P23H RHO allele without significantly affecting the expression of the WT RHO allele. The P23H mutation causes a nonfunctional protein with a toxic gain of function and a dominant negative effect on normal protein. Therefore, reduction of the mutant protein, while sparing the RHO WT protein, should diminish the rate of degeneration associated with adRP and preserve vision for a longer period of time.

### **11:57 AN EX VIVO GENE THERAPY APPROACH IN THE TREATMENT OF X-LINKED**

**RETINOSCHISIS.** KEVIN GREGORY-EVANS, EMRAN BASHAR, ISHAQ VIRINGIPURAMPEER, ANDREW METCALFE. *Vancouver, BC, Canada.*

**Introduction:** As a relatively common cause of juvenile macular degeneration, there is much interest in developing new therapeutics for X-linked retinoschisis. Clinical trials for in vivo gene therapy for this condition are imminent. However, very recent data showing significant limitations for in vivo gene therapy in the eye (N Engl J Med. 2015;372:1887-97) suggest that there is great potential for alternative approaches in therapy. We are developing an ex vivo therapy approach that potentially avoids many of the limitation of in vivo gene therapy.

**Materials & Methods:** Using the RS1KO murine model of X-linked retinoschisis, we obtained mesenchymal stem cells from littermate inguinal fat. These were cultured ex vivo and transfected via electrophoresis with an inducible plasmid designed to express retinoschisin. Expression was induced by exposure to doxycycline. These modified cells were then used in vivo, in studies in the RS1KO murine model treated with topical doxycycline. For up to 8 weeks after intravitreal injection, animals underwent electroretinography (ERG) and optokinetic tracking (OKT). Eyes were then collected for histology and to assess retinoschisin levels in retinal tissue.

**Results:** In vitro experiments with inducible mesenchymal stem cells confirmed expression of retinoschisin and secretion of the protein into culture media supernatant (70-120 ng/10<sup>6</sup> cells/24 hours). Both ERG (b/a ratio) and OKT recording showed improvement with treatment. Histological assessment showed some migration of MSCs into the outer retina and a marked, progressive reduction in the size of schisis cavities over time. ELISA assay demonstrated negligible retinoschisin protein in retina in untreated animals or treated animals without topical doxycycline. This increased to 10pg/100<sup>μ</sup>g at 2 weeks with treatment and exposure to doxycycline, reducing to 4pg/100<sup>μ</sup>g at 8 weeks.

**Conclusions:** We have developed an ex vivo gene therapy approach that shows significant histological and functional improvement in a murine model of X-linked retinoschisis. Added to this are significant safety benefits with less risk of immunogenicity and an inducible system which allows us to control retinal retinoschisin dosage with topical doxycycline.

**12:09 VERSICAN ENHANCES VIRAL-MEDIATED GENE THERAPY BY ACTIVATING JAK/STAT SIGNALING.** RICHARD HURWITZ, PATRICIA AKINFENWA, WESLEY BOND, CRISTHIAN ILDEFONSO, MARY HURWITZ. *Houston, Texas.*

**Introduction:** Gene therapy using either adeno-associated (AAV) or adenoviral (AdV) vectors has been shown to be effective in the ocular environment. Understanding the mechanisms of this enhancement could benefit gene therapy protocols in general. Vitreous, the gelatinous material filling the posterior eye, enhances the expression of transgenes (TGS) delivered by AdV and AAV. Vitreous has no effect on vector internalization but is associated with increased transgene mRNA levels and mediates enhancement in hyaluronan-dependent and independent-manners (Chaudhuri et al. *Mol Therapy* 2007, Ildefonso et al. *JBC* 2012). We hypothesize that versican, a hyaluronan-binding proteoglycan expressed in vitreous, has a role in both mechanisms. Furthermore, we define an intracellular pathway through which the increased gene expression occurs.

**Materials & Methods:** Versican-containing supernatant (VCS) was produced by culturing versican-secreting ACHN cells or HepG2 cells transfected to transiently express the recombinant versican G1 domain either lacking (G1-) or containing (G1+) the hyaluronan-binding region. Y79 or Weri retinoblastoma cells, or SKNDZ neuroblastoma cells that lack a hyaluronan binding domain were transduced in the presence of vitreous, VCS, or either G1 domain with AdV or AAV vectors delivering a luciferase reporter transgene.

**Results:** Incubation of target cells with vitreous enhanced AdV or AAV gene expression 4-6-fold ( $p < 0.001$ ) when Y79 cells, Weri cells, or SKNDZ cells were used as targets. Boiling vitreous prevented this enhancement. Thus, heat-stable hyaluronan alone was not responsible. Incubation with dasatanib (a Src kinase inhibitor), VCS, G1+, or G1- mimicked the effects of vitreous. Small-molecule inhibition of JAK1/2 and STAT3/5 using ruxolitinib and C188-9, respectively, mitigated the enhancement of transgene expression mediated by vitreous, dasatanib, VCS or either G1 domain, while inhibition of the mTOR pathway using rapamycin or everolimus showed no effect on enhancement. Vitreous treatment enhanced baseline STAT3 phosphorylation ( $p < 0.05$ ).

**Conclusions:** These results support a model in which src kinase activity inhibits AdV or AAV viral vector mediated transgene expression. This inhibition can be overcome by hyaluronan-independent and dependent mechanisms mediated by the G1 domain of versican through



activation of the JAK/STAT signaling pathway. Therefore, utilization of the versican G1 domain could be a tool used to enhance gene therapy protocols.

**13:21 GENOME EDITING WITH CRISPR/CAS9 IN ZEBRAFISH: AN EVOLVING TOOL TO MODEL RARE GENETIC RETINAL DISEASES FOR DRUG DISCOVERY.** JASON BERMAN, SERGEY PRYKHOZHII, SHELBY STEELE PHD, VINOTHKUMAR RAJAN, TENILLE FLEISHMAKER. *Ghent, Belgium*

**Introduction:** The zebrafish is well-established as a versatile vertebrate genetic model for studying human disease. While transgenic technology has provided a useful approach for studying gain-of-function mutations, loss-of-function mutations have been more challenging until the recent application of TALEN and CRISPR/Cas9 genome editing. Using this strategy, deletions, insertions and reporter lines can be easily generated, enabling the zebrafish to serve as a much more comprehensive tool for understanding disease pathogenesis and preclinical drug testing. We have been applying these approaches to study mutations underlying familial exudative vitreoretinopathy (FEVR).

**Materials & Methods:** Transient morpholino-based strategies and genome editing approaches with both TALENs (transcription activator-like effector nucleases) and CRISPR (clustered regularly interspaced short palindromic repeats)/Cas9 were employed to mutate established and novel genes identified in the FRZD4-WNT-beta catenin pathway found to be involved as critical for normal retinal vasculature development. Mutants were validated and retinal vascular imaging was performed by taking advantage of using the tg(fli::eGFP) transgenic fish that have green fluorescent endothelial cells.

**Results:** Using both morpholinos and more permanent genome editing approaches, we were able to replicate clinical features of FEVR in zebrafish retina, demonstrating a causative role for these mutations.

**Conclusions:** CRISPR/Cas9 and TALENs provide the technological approaches needed to effectively enable the zebrafish to serve as a robust animal system for modeling rare human eye diseases, such as FEVR. These genome engineered fish models are now poised to serve as an in vivo preclinical screening platform to identify novel therapeutics that can be directly observed to restore normal retinal vasculature.

**14:33 GENE THERAPY RESEARCH REPORTING TRENDS IN THE USA, UK AND CANADA AND IMPLICATIONS FOR CLINICAL COMMUNICATION.** STEPHANIE KOWAL, SHELLY BENJAMINY, IAN MACDONALD, TANIA BUBELA. *Edmonton, Canada*

**Introduction:** In this presentation, we identify challenges and pose solutions for communications about ocular gene therapy research between patients and clinicians. Ocular gene therapy research teams craft press releases and journal articles to present research findings. Ultimately, however, the ease of online information access and sharing creates conditions for these findings to be repeatedly reinterpreted as they move from press releases to newspapers and on to advocacy organization and patients support group websites. We will present our research on information pathways, illustrating how reports of gene therapy progress, among other retinopathy treatment research, change as they moves through information outlets before finally reaching individual patients. The manner in which the information accuracy and quality morphs, affects communication requirements for clinicians

and genetic councillors helping retinopathy patients choose disease management options within current clinical realities.

**Materials & Methods:** We conducted a media analysis of press releases and traditional news media in Canada, the USA, and the UK to compare what stories were most heavily reported in which regions and how these stories were presented by news outlets. Subsequently, we catalogued regionally, nationally, and internationally focused ocular disease advocacy and support groups and analyzed if and how they passed the same research news on to online users. Finally, we also reviewed existing literature on patient perspectives of gene therapy research to fully understand the implications for clinical communications of genetic ocular disease management.

**Results:** For many diseases, including gene therapy, traditional news media: 1) overstates benefits and often omits risks presented in original press releases; 2) conflates research with treatment or cure; and 3) misrepresents research timelines. Some advocacy and support websites also present overly optimistic representations of research realities while others offer reports that are more balanced. Thus, it is likely that patients who actively seek research news receive overly optimistic reports of gene therapy clinical trials from multiple different online news and health support websites. Additionally, newly diagnosed patients may read many of these reports before meeting with genetic councillors and clinicians about disease management decisions.

**Conclusions:** Our research informs how clinicians can prepare to respond to overly positive online messaging. We suggest using optimism towards the future of gene therapy tempered by evidence to make clear the potential and timelines associated with therapies still in clinical trial phases. Clinicians need to communicate about ocular gene therapy in ways that: 1) attend to patient priorities and concerns; and 2) respond to other easily accessible sources of online information.

**12:45**      **Lunch/Poster Viewing**

**13:45**      **Retinoblastoma Session:**  
**The Debate: Cell of Origin in RB - David Cobrinik vs. Brenda Gallie**  
Moderator: Kevin Gregory-Evans

**14:30**      **Symposium Gene Therapy**  
Moderators: Ian MacDonald and Arlene Drack

**14:30 VALIDATION AND CLINICAL APPLIATION OF A NOVEL MOBILITY TEST TO ASSESS FUNCTIONAL VISION IN PATIENTS WITH INHERITED RETINAL DEGENERATION.** DANIEL CHUNG, SARAH MCCAGUE, JENNIFER WELLMAN, ZI-FAN YU, SATHA THILL. *Philadelphia, PA*  
**Introduction:** This mobility test validation study examined the use of a novel standardized mobility test (MT) as a measure of functional vision in normal sighted individuals and in those with inherited retinal degeneration. We evaluated construct and content validity, reliability, the

tests ability to detect changes in functional vision over time, and the tests ability to detect changes in functional vision after intervention in a gene-based clinical trial.

**Materials & Methods:** For the validation study, 28 subjects with various inherited retinal degeneration including choroideremia, Lebers congenital amaurosis, and autosomal recessive retinitis pigmentosa, and 26 normal sighted controls, age 4 through 39 years, were followed for 3 visits over 1 year. Subjects completed MT, standard visual acuity and visual field tests, and a patient questionnaire at all visits. Twelve unique MT course configurations were developed, identical in the number of turns and obstacles, and were selected randomly to minimize learning effect. We evaluated subjects accuracy and speed on MT at 9 standardized light levels from 1 to 400 lux. Tests were scored by at least 2 trained, independent graders. Subjects were assigned a final score of "pass" if they met accuracy and time cut-offs. MT was used as an endpoint in a phase 1 clinical trial involving AAV-mediated gene therapy for inherited retinal degeneration due to mutations in the RPE65 gene.

**Results:** In the validation study, all control subjects passed all MT attempts at all visits and all light levels, in contrast to low vision subjects, who showed a wide range of performance. Inter- and intra-grader variability assessments showed high correlation.

Additionally, course layout variability assessments indicated both time and accuracy were comparable across courses. In the phase 1 AAV-mediated gene therapy trial for inherited retinal degeneration due to mutation in the RPE65 gene, the MT could demonstrate improved functional vision at lower light intensities after therapeutic intervention.

**Conclusions:** Our study demonstrated both construct and content validity in differentiating low vision from control populations, and in identifying a range of performance in low vision patients. MT could also demonstrate the changes in functional vision over time following the natural history of the disease. Course assessment showed that all 12 courses were equally difficult. QA grading activities showed high reproducibility of results and reliability of this endpoint. MT sensitivity was also able to document improved functional vision at lower light intensities after a gene therapy intervention in a phase 1 trial.

#### **14:50 CRITICAL ANALYSIS OF TESTS SEPARATING ROD AND CONE VISUAL PATHWAYS AS OUTCOME PARAMETERS IN GENE THERAPEUTIC TRIALS OF HEREDITARY RETINAL DISEASES.**

BIRGIT LORENZ. *Giessen, Germany*

**Introduction:** Rod and cone pathways are affected to different degrees in retinal diseases. The value of methods separating rod from cone function will be analyzed in view of gene therapeutic endpoints.

**Materials & Methods:** Comparative analysis of feasibility and results of with global tests such as scotopic and photopic Ganzfeld electroretinogram, fullfield threshold test FST, dark adaptometry, and chromatic pupillometry, and of tests with spatial resolution such as 2-color-threshold perimetry 2CTP, fundus-controlled perimetry and imaging methods (SD-OCT, AO).

**Results:** Published and own data show that so far, electroretinography has been of no value in retinal dystrophies as signals were too low both before and after therapy. FST, dark adaptometry and chromatic pupillometry showed consistent treatment effects as well as 2CTP and fundus controlled perimetry, the latter being compromised by the limited range of sensitivity. Sophisticated analysis of SD-OCT documented progression of atrophy.

**Conclusions:** Changes in rod and cone pathway function and morphology can be monitored successfully with global and spatially resolved tests, and hence can serve as outcome parameters. Applicability of functional tests is limited by patient capacities. Not all outcome parameters are of clinical value for the patient.

**15:10 MANAGING EXPECTATIONS IN GENE THERAPY TRIALS AND LESSONS LEARNED ALONG THE WAY.** ARLENE DRACK. *Iowa City, Iowa*

**Introduction:** The Phase III gene therapy trial for RPE65 LCA being conducted at Children's Hospital of Philadelphia and the University of Iowa enrolled the youngest patients treated thus far with subretinal gene therapy. The process of consenting and testing young children for an experimental treatment will be discussed. We will present for discussion some topics that are rarely addressed such as the expectations of parents and children in clinical trials, how to help patients understand the risks vs benefits of participating in a clinical trial, and the importance of diligent genetic testing when deciding which patients will be enrolled, including the controversy of whether to enroll patients who have only one allele found in an autosomal recessive condition.

**15:30 PARTICIPANT SELECTION FOR A PHASE 1 CLINICAL TRIAL OF AAV2-REP IN CHOROIDEREMIA.** IAN M MACDONALD, IOANNIS S DIMOPOULOS, STEPHANIE CHAN, GARY GOLDSAND, RIZWAN SOMANI. *Edmonton, AB, Canada*

**Introduction:** To define the process for the selection of potential participants for a safety trial of AAV2-REP1 for choroideremia ([clinicaltrials.gov](https://clinicaltrials.gov/ct2/show/study/NCT02077361) NCT02077361)

**Materials & Methods:** Prospective participants who self-identified to the Albert Ocular Gene Therapy Team as being interested in the trial were included in the review process. In addition, the Foundation Fighting Blindness Canada Patient Registry was accessed to review potential candidates from across Canada. The following inclusion criteria were considered: a) BCVA equal to or worse than 20/30 but better than or equal to 20/200 in the study eye and b) expectation of significant visual function decline over the subsequent 5 years with OCT changes visible within the macula. In total 20 subjects were identified; all were from Canada and had recruited as an external board to review clinical data from these subjects. The surgeons were provided with the following de-identified information: age, vision, fundus photography including autofluorescence and optical coherence tomographic images, but masked as to the origin of the patients.

**Results:** Six candidates who met the inclusion criteria and for whom there was the highest concordance between the surgeons were carefully selected. High-priority candidates were characterized by the presence of small foveal (<3 mm<sup>2</sup>) or large extrafoveal islands of residual fundus autofluorescence with reduced microperimetry readings. All these patients exhibited mixed cone-rod or cone-mediated perception, based on the difference between blue and red stimulus FST thresholds (Roman et al. 2005). Patients with microperimetric retinal sensitivity at the CHM-adjusted ceiling were considered of low priority, even though BCVA was within the inclusion criteria range. These candidates exhibited rod-mediated perception and had residual FAF islands > 4 mm<sup>2</sup>. Patients with OCT-evident splitting of the fovea by the degeneration were excluded.

**Conclusions:** Patient selection for a phase 1/2 CHM gene therapy trial should be guided by OCT

and microperimetry and not solely rely on BCVA. Ideal candidates should be able to exhibit gain in retinal function without the risk of losing vision from the surgical procedure.

Enrolling patients too early in the disease stage or at the ceiling of retinal sensitivity carries a higher risk/benefit ratio. A process was defined that removed selection bias for participant selection in the trial of ocular gene therapy. The process could be shared with patients, funders and colleagues to demonstrate that careful consideration had been given to selection of patients prior to the consent process.

**15:50 UPDATE ON RETINAL GENE THERAPY TRIALS AT THE CASEY EYE INSTITUTE.** MARK PENNESI, RICHARD G. WELEBER, ANDREAS K. LAUER, PAUL YANG, JOSE A. SAHEL. *Portland, Oregon*

**Introduction:** The field of retinal gene therapy is expanding as new treatments are being developed for inherited retinal dystrophies. The Casey Eye Institute is currently participating in five gene therapy trials the follow diseases: Leber Congenital Amaurosis Type 2 due to RPE65 mutations, ABCA4-related retinopathy, Usher syndrome type 1B, X-linked retinoschisis, and achromatopsia due to CNGB3 mutations.

**Materials & Methods:** Twelve patients with genetically confirmed LCA Type 2 were treated with rAAV2-CB-hRPE65 in the sub-retinal space as part of a Phase I/II trial sponsored by AGTC. Sixteen patients with genetically confirmed ABCA4-related retinopathy were treated with SAR422459, an EIAV based vector, as part of a Phase I/II trial sponsored by Sanofi. Five patients with genetically confirmed MYO7A mutations were treated with Ushstat, an EIAV based vector, as part of a Phase I/II trial sponsored by Sanofi. A Phase I/II trial sponsored by AGTC using intravitreally delivered rAAV2tYf-CB-hRS1 for XLRS has commenced and a similar trial for Achromatopsia is being planned. Safety and efficacy assessments include : visual acuity, static and kinetic visual fields, full field and multifocal ERGs, fundus photography, autofluorescence, and SD-OCT.

**Results:** Treatments with various retinal gene therapies have been well tolerated without significant adverse effects. Evaluations for biological efficacy are underway.

**Conclusions:** Since the first human gene therapy trials in 2008, the number of gene therapy trials continues to expand.

**16:10 CURRENT THINKING ABOUT LONGEVITY OF THE EFFECT OF SUBRETINAL GENE THERAPY FOR RPE65-RELATED LCA.** BART P LEROY. *Ghent Belgium*

**Introduction:** To discuss the data available in the literature on the longevity of the effect of gene therapy for RPE65-related LCA (LCA2).

**Materials & Methods:** Data from the literature will be combined with longitudinal follow-up data from two patients from Ghent, who participated in the Phase 1 and Phase 1 add-on trial at the Children's Hospital of Philadelphia, Philadelphia, PA, USA.

**Results:** According to some recent study results, there is progression of disease in treated LCA2 patients, despite successful subretinal delivery of RPE65 using an AAV2 vector. Other data suggest that there may at least be a decrease in the speed of retinal degeneration, if not stabilization.

**Conclusions:** Whereas disease progression is noted in some LCA2 patients, despite successful application of subretinal gene therapy, others may even have a stable disease course after treatment.

**16:30**      **Poster Viewing**

**17:00**      **Retina Degenerative Disease Patient and Family Session**

*Sponsored by Foundation Fighting Blindness, Canada*

Panel of speakers on a variety of topics including basics of genetics, role of genetic testing and new technologies, gene therapy and gene-based therapies

**19:00**      **Gala Dinner Maritime Museum reception and visit**

## POSTERS

**Tania Barragan Arevalo (Mexico)**

Novel ACTG1 mutation in a Mexican patient with Baraitser-Winter syndrome

**Miriam Ehrenberg (Israel)**

NCF2-related chronic granulomatous disease and retinitis pigmentosa

**Mary Hurwitz (USA)**

Decreased expression of SKAP2 increases proliferation of retinoblastoma cells

**Timothy Corson (USA)**

Residual disease monitoring in a retinoblastoma patient by PCR of a novel deletion breakpoint

**Mohamed H. Abdel-Rahman (USA)**

Comprehensive review of the BAP1 tumor predisposition syndrome with report of three new cases

**Justin Pyne (Canada)**

Visual outcomes in carriers of familial exudative vitreoretinopathy (FEVR) gene mutations

**Michael Ngo (Canada)**

FZD4 haploinsufficiency delays recovery of retinopathy of prematurity in the ocular ischemic retinopathy (OIR) mouse model

**Dianna Highbanks-Wheaton (USA)**

Retinal targeted-capture next generation sequencing and CLIA confirmation in a representative range of patients with inherited retinal degeneration: a pilot of the Texas 1000 project.

**Hoda Rajabi (Canada)**

Validation of a diagnostic panel comprised of ABCA4 mutations causing Stargardt disease in the Newfoundland and Labrador (NL) population

**Rajani Battu (India)**

Status of genetic eye research in India: An analysis of the publications in eye genetics in the last decade and the challenges associated

**Heather MacDonald (Canada)**

Phenotypic characterization of males with suspected X-linked juvenile retinoschisis is highly sensitive to detect RS1 mutations

**Sandra Staffieri (Australia)**

Clinical and molecular characterization of females affected by X-linked retinoschisis

**Hannah Scanga (USA)**

X-linked juvenile retinoschisis with manifestations in female carriers demonstrated by ophthalmic imaging

**Choi Mun Chan (Singapore)**

A family with maternally inherited diabetes and deafness (MIDD) from Singapore

**Kari Branham (USA)**

Integrating Genetic Testing into a Retinal Dystrophy Clinic



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Thursday – 6.75

Friday – 5.75

Saturday – 8.25

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## ISGEDR Travel Awards

Razek Coussa, Medical Student, McGill University, *Montreal, Canada*

Susan Wakil, Ophthalmology Resident, McGill University, *Montreal, Canada*

Miriam Ehrenberg, M.D., Pediatric Ophthalmologist, *Tel Aviv, Israel*

Rajani Battu, M.D., Pediatric Retina Specialist, *Bangalore, India*

## Spark Therapeutics Genetic Counselor Travel Award

Hannah Scanga, Genetic Counselor, Pittsburgh, USA