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# 4<sup>TH</sup> EUROPEAN CONFERENCE ON ANIRIDIA, IRIS AND CORNEA DEVELOPMENTAL ANOMALIES

**PARIS, FRANCE**

**CISP RAVEL**  
6 avenue Maurice Ravel  
75012 – PARIS  
Métro : L1 Porte de Vincennes / L6 Bel Air  
Tram T3A – Alexandra David-Néel/ Montempoivre

ASSOCIATION  
**Geniris**  
Aniridia et pathologies rares de l'iris  
Avec ou sans syndromes associés



Lions club Saint Pol en Ternois



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## @ CONTACTS



Tél : +33(0)658297527 - Email : [associationgeniris@gmail.com](mailto:associationgeniris@gmail.com)

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**Centre de Référence de Maladies RAres en OPHTAlmologie**

**– CHU NECKER Enfants Malades – Paris – France**

**Tél : +33(0)144494502**

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**SESSION 1 :**  
**UPDATE IN GENETICS**  
**IN ANIRIDIA**

## **Dr Patrick Callaerts (Belgium)**

Patrick Callaerts was trained as a biologist at University of Leuven where he also obtained a PhD in Biology. During his postdoctoral studies with Walter Gehring in Basel (Switzerland) he performed seminal experiments on the role of Pax6 as master regulator of eye development. He was faculty in the Department of Biology and Biochemistry at University of Houston from 1997 until 2004. He was appointed professor at KU Leuven in 2004 and heads the Laboratory of Behavioral and Developmental Genetics.



### **« PAX6 : what we have learned ? »**

The genetic cause of congenital Aniridia was identified in 1991 with the cloning of the gene PAX6 encoding a transcription factor with DNA-binding paired and homeodomains. In a rapid succession of discoveries, it was subsequently shown that PAX6 is conserved throughout evolution and is in many cases involved in eye development. Work of the past decades has resulted in unprecedented insight in how eyes develop. In addition, our growing understanding of the genetic regulatory networks involved has contributed to the identification of mechanisms that regulate transcription factor activity and of genetic causes of eye disease. Our current insight and future challenges will be discussed.

## Pr Giuseppe Damante (Italy)

MD, PhD

Degree in Medicine and Surgery in 1982 at Catania University. From 1985 to 1987, fellow at University of California – San Francisco. From 1988 to 1990, PhD position at European Molecular Biology Laboratory, Heidelberg. From 1990 to 1998, research position Udine University. From 1998 to the present, professor of Medical Genetics at Udine University and Director of the Medical Genetics Institute at the University Hospital of Udine. Interested in the molecular genetics of aniridia since 2001.



### "Screening of PAX6 gene in Italian congenital aniridia patients"

Alessandra Franzoni<sup>1</sup>, Catia Mio<sup>2</sup>, Federica Baldan<sup>2</sup>, Lucia Mauri<sup>3</sup>, Alessandra Del Longo<sup>3</sup>, Elena Piozzi<sup>4</sup>, Emanuela Manfredini<sup>3</sup>, Shipra Bhatia<sup>5</sup>, Giuseppe Damante<sup>1,2</sup>,

<sup>1</sup> Istituto di Genetica Medica, Azienda Sanitaria Universitaria Integrata di Udine, Italy

<sup>2</sup> Dipartimento di Scienze Mediche e Biologiche – Università di Udine, Italy

<sup>3</sup> Dipartimento di Medicina di Laboratorio, SS Genetica Medica - ASST Grande Ospedale Metropolitano Niguarda, Milano, Italy

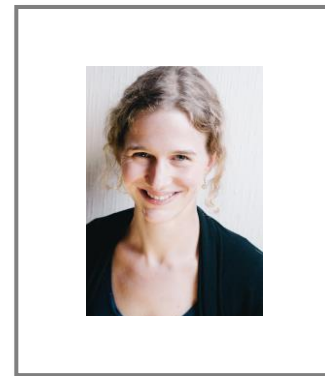
<sup>4</sup> Dipartimento Materno Infantile, S.C. Oculistica Pediatrica – ASST Grande Ospedale Metropolitano Niguarda, Milano, Italy

<sup>5</sup> Human Genetics Unit, IGMM, Edinburgh, United Kingdom

Aniridia is a rare congenital disease of the eye in which there is a variable degree of hypoplasia or the absence of iris tissue associated with multiple other ocular changes. Most cases are associated with dominantly inherited mutations or deletions of the *PAX6* gene. Large deletions of *PAX6* region and involving *WT1* gene give rise to WAGR syndrome. The present study includes 138 and 3 Italian patients affected by aniridia and WAGR syndrome, respectively. Among aniridia cases, 65 were familial and 73 sporadic; all WAGR subjects were sporadic. Point mutations or small deletions of the *PAX6* gene were detected by Sanger sequencing and MLPA analysis. Large deletions of the *PAX6* genomic region were detected by CGH array. In all three WAGR patients, large deletions including the *PAX6* gene were detected. Among aniridia subjects causative point mutations or deletions have been found in 75 patients (54%): 10 indel, 7 missense, 22 nonsense, 12 splicing, 24 deletions. The DNA-binding and transactivation properties of two missense mutations have been analyzed by gel-retardation and reporter assays. Interestingly, in 6 patients no mutation was found at the level of *PAX6* gene, but a deletion in the *ELP4* gene was present. By CGH array we demonstrated that these deletions always include the SIMO enhancer of *PAX6* gene. In addition, in a single patient a de novo point mutation in the SIMO enhancer was found. Experiments in zebrafish indicated that this mutation abolishes the enhancer activity of the SIMO element, therefore affecting *PAX6* expression. Our data, therefore, support the notion that aniridia can be caused by alteration of transcriptional control elements of *PAX6* gene.

## Dr Nikki Hall (UK)

Nikki Hall moved from Brussels to Edinburgh in 2003 to study medicine. She started specialist training in Ophthalmology in 2011, becoming a Fellow of the Royal College of Ophthalmologists in 2016, and holds a clinical academic post as a Clinical Lecturer at the University of Edinburgh. In 2016, she joined the lab of Prof David FitzPatrick for a Wellcome Trust-funded PhD investigating the genetic and development basis of PAX6-negative aniridia.



### « Genetic Analysis of ‘PAX6-Negative’ Individuals with Aniridia »

H. Nikki Hall<sup>1</sup>, Morad Ansari<sup>1</sup>, Kathy A. Williamson<sup>1</sup>, Shipra Bhatia<sup>1</sup>, Hemant Bengani<sup>1</sup>, Veronica van Heyningen<sup>2</sup>, David R. FitzPatrick<sup>1</sup>

<sup>1</sup> MRC Human Genetics Unit, MRC Institute of Genetics & Molecular Medicine, University of Edinburgh, United Kingdom; <sup>2</sup> Institute of Ophthalmology, London, United Kingdom

Identifiable heterozygous mutation or deletion of the *PAX6* locus accounts for approximately 90% of aniridia cases. This includes mutations in *cis*-regulatory regions of *PAX6*. Non-*PAX6* causes of isolated aniridia include - rarely - mutation or deletion of *FOXC1* or *PITX2*, loci which more commonly cause anterior segment dysgeneses. Aniridia is a clinical feature of several genetically-distinct conditions: syndromic aniridia that is not *PAX6*-related may be caused by *ITPR1* mutations, recently identified as the causal gene in Gillespie syndrome, and *ACTA2* mutations, which are associated with multisystemic smooth muscle dysfunction syndrome. The distinctive iris phenotype in these two conditions is characterised by absence of the iris sphincter muscle. We outline a suggested clinical investigation strategy for aniridia, detailing an approach based on chromosomal array and gene panel testing. This is guided by the iris phenotype and aims to test all known aniridia loci, including the rarer, life-limiting causes such as *ACTA2* mutations when appropriate. Lastly, at least 5% aniridia cases remain unexplained. Aiming to address this, we discuss our ongoing whole genome sequencing study of a *PAX6*-negative aniridia cohort (n=50).

## Pr Tatiana Vasilyeva (Russia)

Scientific associate, Laboratory of Genetic Epidemiology, Research Centre for Medical Genetics, received M.Sc. from Lomonosov Moscow State University in 1985, Ph.D. from Research Centre for Medical Genetics in 2018 (under supervision of Dr. A.V.Marakhonov). Scientific interests include medical genetics, molecular diagnosis, and molecular pathogenesis of hereditary diseases.



### “Analysis of genotype–phenotype correlations in aniridia in Russian federation”

T.A. Vasilyeva<sup>1</sup>, A.A. Voskresenskaya<sup>2</sup>, B. Käsman-Kellner<sup>3</sup>, N.A. Pozdeyeva<sup>2</sup>, A.V. Marakhonov<sup>1,4</sup>, R.A. Zinchenko<sup>1,5</sup>

<sup>1</sup> Federal State Budgetary Institution «Research Center for Medical Genetics», Moscow, Russia

<sup>2</sup> Cheboksary branch of S. Fyodorov Eye Microsurgery Federal State Institution, Cheboksary, Russia

<sup>3</sup> Kinderophthalmologie, Orthoptik, Low Vision, Neuroophthalmologie, Landesblindenärztin des Saarlandes, Homburg, Germany

<sup>4</sup> Moscow Institute of Physics and Technology (State University), Moscow, Russia

<sup>5</sup> Pirogov Russian National Research Medical University, Moscow, Russia

Congenital aniridia is an autosomal dominant severe panocular disorder caused by mutations in *PAX6* gene as well as large chromosomal aberrations affecting 11p13 chromosomal region. Despite several attempts to establish genotype-phenotype correlations in aniridia to date no association between diverse clinical signs of congenital aniridia and type of *PAX6* damage were revealed. Recently we have performed clinical and molecular genetic analysis of 110 patients with congenital aniridia from Russia [1]. 3 patients reveal no changes affecting *PAX6* gene or 11p13 chromosomal region. Such a numerous cohort of patients with identified genetic cause allows us to analyze genotype–phenotype correlations in aniridia. In order to get uniformly examined and well-established cohort of patients, 3 of them lacking detailed ophthalmological assessment were excluded from further analysis. 6 more patients with aniridia were ruled out because of identified *WAGR* region deletions. This resulted in cohort of 98 patients from 73 unrelated families (44 sporadic and 54 familial). Mean age is 16 years old (range from 6 month to 65 years old). Sex ratio is 1:1.3.

Observed eye anomalies include complete or partial aniridia, nystagmus, keratopathy, cataract, glaucoma, and foveal hypoplasia. Genetic causes are divided into six types: nonsense, missense, splicing, frame-shifting insertions/deletions in *PAX6* gene, large chromosome rearrangements (excluding ones affecting 3'-*cis*-regulatory region), and 3'-*cis*-regulatory region deletions in 11p13. Two-sided Fisher's exact test is applied to the 2×2 contingency tables of clinical sign and mutation type.

Our results reveal that complete absence of iris and keratopathy are predominantly associated with nonsense mutations ( $p=0.002923$  and  $p=0.040567$ , respectively). Patients with missense mutations significantly more often develop partial aniridia ( $p=0.021778$ ). Frame-shifting mutations are frequently associated with cataract and glaucoma ( $p=0.035935$ , and  $p=0.02488$ , respectively). Patients with 3'-*cis*-regulatory region usually develop a milder aniridia phenotypes without nystagmus, keratopathy, glaucoma or foveal hypoplasia ( $p=0.040246$ ,  $p=0.000418$ ,  $p=0.007655$ , and  $p=0.00074$ , respectively). Phenotypes of patients with large chromosomal rearrangements (excluding 3'-*cis*-regulatory region deletions) do not significantly differ from those with point mutations in *PAX6* gene ( $p>0.1$ ).

To our best knowledge, here is the first report of associations between *PAX6* damage types and ocular peculiarities in aniridia. That could be possible in consequence of tight professional engagement and interaction between molecular and ophthalmogeneticists.

This work was supported by the Russian Scientific Foundation grant № 17-04-00475.

Reference : 1 Vasilyeva T.A. et al., Molecular analysis of patients with aniridia in Russian Federation broadens the spectrum of *PAX6* mutations. Clin Genet. 2017 Mar 21. doi: 10.1111/cge.13019.



## Pr Patrick Calvas (France)

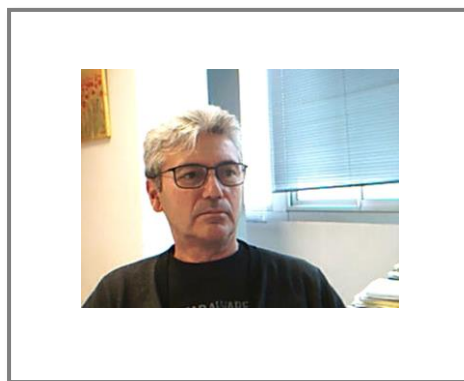
MD, PhD

Professor of Medical Genetics at the Université de Toulouse UPS-Toulouse III)

Head of the Medical Genetics Department in Toulouse University Hospital

Research group leader on Ocular Developmental Defects at INSERM/UPS UMR1056-UDEAR- Toulouse (France)

Vice-Chair of the French Human Genetics Society (SFGH)



### « PAX6 unusual mutations and phenotypes » -

Aniridia is a panocular disorder mainly characterized by the absence of part or all of the iris. The canonical phenotype is associated with dominant heterozygous *PAX6* mutations while some rarer cases are related to variant of other genes. Around 80% of *PAX6* mutant alleles leading to classical aniridia consist in premature STOP codons commonly leading to haploinsufficiency through non-sense mediated mRNA decay. For a long time literature reported variant ocular phenotype associated with less common ocular phenotype such as Peters anomaly or Axenfeld-Rieger anomaly optic disc dysplasia, or milder defects such as high myopia.

We report a series of unusual mutations leading to various defects and provide the corresponding phenotypes thus extending both phenotypic spectrum of the aniridia complex and the high frequency of mutations missed by routine screening procedure.

We will bring rare cases of compound heterozygosity associated with anophthalmia, variable dysmorphic features, severe central nervous system malformations including holoprosencephaly corpus callosum agenesis, vermis hypoplasia, heterotopias, pyramidal tract aplasia. In addition we will try to enlight the reason why series of contiguous heterozygous missense mutations located in the region encoding the paired domain are linked to severe anomalies such anophthalmia and cerebral malformations. We will present the preliminary *in vitro* experiments questioning the nature and diversity of the causative mechanisms.

On the opposite we will bring example of milder phenotypes linked to somatic mosaicism of the mutant allele.

Finally we will expose the results of a recent study of the non-coding regions (untranslated regions, introns and *cis*-regulatory sequences) conducted in patients suffering classical aniridia with no variant or del/dup event found in or around the gene. Surprisingly candidate variants were evidenced in 40% of the patients while functional studies made most of them strong causative mutations.

Such analysis dramatically improved the molecular diagnosis scheme in aniridia patients and confirm that *PAX6* mutations are responsible of more than 90-95% cases in aniridia patients.

## Pr Elena V. Semina (USA)

Dr. Elena V. Semina is the Chief of the Division of Developmental Biology within the Department of Pediatrics and a Professor in the Departments of Pediatrics, Ophthalmology and Visual Sciences, and Cell Biology, Neurobiology, and Anatomy (CBNA) at the Medical College of Wisconsin. Dr. Semina has significant expertise in vision research with over 90 publications and continuous NIH funding since 2002. She serves as an Ad Hoc Reviewer for numerous granting agencies, on the Editorial Board of *Molecular Vision*, and as Vice President, Americas, for the International Society for Eye Research (ISER). Dr. Semina has mentored over 50 undergraduate, graduate, and postdoctoral students at MCW. She is a member of the Executive Evaluations Committee, regularly teaches PhD and MD students, and has been a member of many PhD thesis committees.



### “Genetic heterogeneity in aniridia phenotype”

Elena V Semina (Medical College of Wisconsin, Milwaukee, WI 53226, USA)

Aniridia is a rare ocular disorder affecting the iris, cornea, lens, retina, macula and optic nerve. Approximately 2/3 of aniridia cases are familial with typically an autosomal-dominant pattern of inheritance and variable expressivity. Mutations in *PAX6* gene explain the majority of aniridia cases (60-97%) in different published reports and include intragenic alleles, partial/whole coding region deletions, or regulatory mutations. At the same time, *PAX6*-negative cases have been described by several papers and studies into their molecular mechanisms are ongoing. Through our genetic program in developmental ocular disorders, we identified 28 families diagnosed with aniridia and lacking pathogenic alleles in *PAX6*. Five of these patients were found to have causative mutations in forkhead transcription factor *FOXC1* (both deletions and intragenic), two- pathogenic alleles in homeodomain transcription factor *PITX2* (whole gene deletion and intragenic), two- in forkhead transcription factor *FOXE3*, and one- in a retinoic acid receptor beta *RARB*. Whole exome sequencing of other unexplained cases identified several variants of unknown significance in genes that need to be further explored for their possible role in aniridia.

**SESSION 2 :**  
**SYSTEMIC DEFICIENCY**  
**RELATED TO ANIRIDIA**

## **Dr Martin Fredensborg Rath (Denmark)**

**Affiliation:** Department of Neuroscience, Faculty of Health and medical Sciences, University of Copenhagen, Copenhagen, Denmark.

PhD

Martin Fredensborg Rath is Associate Professor at the University of Copenhagen. Dr. Rath received his PhD in Neuroscience in 2009. He did pre- and postdoctoral training with Prof. Morten Møller (University of Copenhagen, Denmark) and Dr. David C. Klein (National Institutes of Health, MD, USA). In a combined effort involving neuroanatomical and molecular biological techniques, Dr. Rath and his group investigate development and adult function of the circadian system of the brain.



### **“The role of Pax6 and other ocular homeobox genes in circadian biology of the pineal gland”**

The pineal gland is a neuroendocrine gland responsible for nocturnal synthesis of melatonin. Like the retina of the eye, the pineal gland develops as an evagination from the diencephalon. In non-mammalian vertebrates, the pineal gland is a light-sensing organ referred to as the third eye.

In both the eye and the pineal gland, homeobox gene-encoded transcription factors of the paired box (Pax)- and orthodenticle homeobox (Otx)-families are expressed and are essential for normal eye and pineal development consistent with the well-established role that homeobox genes play in developmental processes. However, the pineal gland appears to be unusual because strong homeobox gene expression persists in the pineal gland of the adult brain. Accordingly, in addition to developmental functions, homeobox genes appear to be key regulators in postnatal phenotype maintenance in this tissue. The presented work will focus on ontogenetic aspects of pineal gland development and recent progress in understanding the involvement of homeobox genes in pineal development and adult function. A working model is proposed for understanding the sequential action of homeobox genes in controlling development and mature circadian function of the mammalian pinealocyte, the main cell type of the pineal gland, based on knowledge from detailed developmental and daily gene expression analyses, pineal phenotypes of knock out mice, siRNA studies on cultured pinealocytes, and studies on development of the eye. The pinealocyte and retinal photoreceptor share features not seen in other tissues and are likely to have evolved from the same ancestral photodetector cell. Comparative analyses of homeobox gene expression in the pineal gland and the eye will be presented.

## Dr Nadia Bahi-Buisson (France)

MD PhD

N. Bahi-Buisson is Professor, Department of Pediatric Neurology, Necker Enfants Malades Hospital, and group leader in the team of A Pierani at the Imagine Institute INSERM UMR-1163 Paris Descartes University, Paris. She coordinates a group of research on MCDs with a Post Doc fellow (Amandine Bery) and 2 PhD students (S Farcy and C Maillard). She obtained an ANR grant in 2016, aiming to elucidate the variable phenotypes associated with DYNC1H1 mutations.

In November 2017, she obtained a "contrat d'Interface INSERM" for the project on «Molecular and cellular basis of cortical malformations » in order to devote her full time to research during a period of at least 3 years. The group is interested in elucidating genetic causes of human MCD. N.Bahi-Buisson formerly a member of Chelly's group at Cochin Institute – INSERM U1016 Paris, has developed a unique experience in clinical and genetic characterization of MCDs, giving a significant contribution to this field.

More recently, her group decided to work on neurodevelopmental aspect in aniridia, in close collaboration with Pr Bremont Gignac in Necker. Her group interacts closely with Pr P Calvas and Dr N Chassaing, members of the « Reference Centre for Eye defects ». She also participates as pediatric neurologist expert in the cohort follow-up, Radico-oeil.



### **“Neuropaediatrics and aniridia : diagnostic and rehabilitation”**

Aniridia is a severe, congenital ocular malformation inherited in an autosomal-dominant fashion with high penetrance and variable expression. Eye morphogenesis involves a molecular genetic cascade in which a number of developmental genes interact in a highly organized process during the embryonic period to produce functional ocular structures.

There are growing evidences that aniridia patients may have non-ocular sensory and neurological deficits. In patients with isolated aniridia, reduced olfaction appears to be the most common functional deficit. Moreover, congenitally blind children are generally reported to be at risk for serious behavioral and psychological problems, such as withdrawal, isolation, and autism. Nevertheless, in aniridia, cognitive function is generally normal and behavioural difficulties and developmental delay are rare.

Among gene involved in aniridia, paired box gene 6 (PAX6) has an essential role as it encodes a phylogenetically conserved transcription factor almost universally employed for eye formation in animals with bilateral symmetry, despite widely different embryological origins. Aniridia occurs due to decreased dosage of the PAX6 gene and exists in both sporadic and familial forms. In the vast majority of cases, aniridia is caused by the loss of function of one copy (haploinsufficiency) of the PAX6 gene. The PAX6 gene encodes a transcription factor with multiple roles in the development of the eye and other tissues. Familial aniridia cases show autosomal dominant inheritance with high penetrance but considerable phenotypic heterogeneity. In some cases, autism and intellectual disabilities may occur, with a significant alteration of quality of life.

The second condition in which aniridia is associated with neurodevelopmental disorder is the Gillespie syndrome, a rare variant form of aniridia characterized by non-progressive cerebellar ataxia, intellectual disability, and iris hypoplasia. Recently, the genetic basis were identified with mutations in ITPR1 a member of the IP3-receptors family that form Ca(2+) release channels localized predominantly in membranes of endoplasmic reticulum Ca(2+) stores.

Evaluating the neurodevelopment in a patient with aniridia is challenging. First, we recommend that mostly trained pediatric neurologist examine the child and give his opinion to identify subtle signs. Motor, speech and social behavior have also to be tested

If a screener indicates that a child may have autism spectrum disorder, the child should receive a comprehensive evaluation from someone trained in diagnosing autism, although no autism diagnostic scale are valid .

The most important factors that most likely account for the comorbidity of autistic disorder in blind children are severity of brain damage and intellectual disability. Therefore, in addition to treatment and rehabilitation of their ophthalmological and neurological problems, these children need early intervention programs for their socio-emotional development, language and behavioral problems.

## **Pr Dominique Bremond-Gignac (France)**

Head of Ophthalmology Department  
University Hospital Necker Enfants-malades, APHP,  
149 rue de Sèvres, 75015 PARIS, FRANCE  
CNRS Unit FR3636, University Paris V René Descartes  
Head of Rare Eye diseases Center OPHTARA, member  
ERN EYE  
Head of Clinical Research Center CLAIROP  
[dominique.bremond@aphp.fr](mailto:dominique.bremond@aphp.fr)



MD, PhD

Dominique BREMOND-GIGNAC is Professor of Ophthalmology Head of Ophthalmology Department with pediatric subspecialty at University Hospital Necker-Enfants malades and Paris V Rene Descartes University in Paris. Head of Paris V Orthoptic Department, affiliated to the CNRS FR3636 Research Unit, in binocular vision. Her activity is distributed in clinical practice, teaching and research (eye development). Current practice includes pediatric anterior segment, strabismus and oculo-plastic surgery as she is also graduated in maxillo-facial surgery. She contributed over a hundred peer review publications in the ophthalmic literature and more than fifty book chapters. Involved in visual health in children she is Executive member of WSPOS (World Strabismus and Pediatric Ophthalmology Society). Expert for the French Health and Higher Education Ministries about Orthoptic training. Head of CLAIROP Research Clinical Center accredited by Europe EVI-CT and Head of OPHTARA Rare Eye Diseases Center accredited by French Health Ministry and ERN EYE (accredited by Europe).

### **“Phenotype-Genotype correlation in aniridia”**

Congenital aniridia consists in a complex malformation of the eye with congenital absence of iris. Aniridia is a rare panocular disorder affecting, beyond iris, cornea, angle structures, lens and fovea, and possibly associated with other anomalies. This genetic rare disease can cause severe visual impairment occurring from various mechanisms as glaucoma, limbal insufficiency and foveal hypoplasia. PAX6 gene is mainly involved in the disease. We aim to understand the features of patients with congenital aniridia, identifying the characteristics phenotypes of the disease and its associated anomalies. We analyse our patient cohort who underwent a complete ocular examination with ophthalmic exploration and who had genetic molecular analysis. Specific analysis of each ocular feature (as aniridia-related keratopathy, glaucoma, cataract, foveal hypoplasia, optic nerve hypoplasia and other features) is detailed with early clinical signs that can be observed. An evaluation of risk factors is required for the follow-up of aniridia patients in order to get a better therapeutic orientation.

**SESSION 3 :**  
**SYNDROMIC ANIRIDIA**

## Dr Lucas Fares Taie (France)

Ph.D

Lucas FARES TAIE, obtained his degree in Biochemistry and a Master of Science (M.Sc) in Medical Molecular Biology, from the University of Buenos Aires in Argentina. He obtained a Ph.D. in Molecular and Cell Biology from the University of Paris Descartes, working on the characterization of the molecular and physiopathological bases of severe ocular defects. After completing his doctoral studies, he continued in this interesting and challenging field at the Institute of rare genetic diseases at Imagine where he identified the genes for three emblematic eye dysgenesis namely MicroAnophthalmia, Congenital Microcoria and Gillespie Syndrome.



### « Recessive and Dominant De Novo *ITPR1* Mutations Cause Gillespie Syndrome »

Fares Taie L1, Gerber S1, Alzayady KJ2, Burglen L3, Brémond-Gignac D4, Marchesin V5, Roche O4, Rio M6, Funalot B7, Calmon R8, Durr A9, Gil-da-Silva-Lopes VL10, Ribeiro Bittar MF10, Orssaud C4, Héron B11, Ayoub E2, Berquin P12, Bahi-Buisson N13, Bole C14, Masson C15, Munnich A6, Simons M5, Delous M16, Dollfus H17, Boddaert N8, Lyonnet S18, Kaplan J1, Calvas P19, Yule DI2, Rozet JM20.

Gillespie Syndrome (GS) is a very rare variant form of aniridia characterized by scalloping of the pupillary edge and the presence of iris strands extending onto the anterior lens surface. This iris hypoplasia is associated with infantile cerebellar ataxia and intellectual disability. Recently, it has been demonstrated that biallelic and monoallelic *ITPR1* mutations are responsible for the molecular defect underlying Gillespie Syndrome confirming the long-suspected coexistence of autosomal-recessive or dominant patterns of inheritance. This gene encodes an IP3-dependent calcium-release channel which plays a key role in the regulation of intracellular calcium concentration. Sporadic GS affected individuals showed that the de novo mutations located in the distal portion of the sixth membrane domain of the channel contributing to the pore result in a dominant-negative effect. Inversely, the biallelic mutation are expected to produce truncating proteins lacking the channel domain. These results suggest that the mutations that affect the channel pore and thus the gating might be more severe than those which affect the regulation of the channel activity. Our study contributes further to the description of the pathognomonic sign of aniridia associated with *ITPR1* mutations in Gillespie Syndrome individuals, which should be used to direct the genetic studies of this gene accounting for 62 exons. Finally, we suggest that the residual channel activity is correlated with the severity of the disease which ranges from adult onset spinocerebellar ataxia in individuals with 50% of *ITPR1* activity to Gillespie syndrome in patients with low, but not absent, *ITPR1* activity.

1Laboratory of Genetics in Ophthalmology (LGO), INSERM UMR1163, Imagine – Institute of Genetic Diseases, Paris Descartes University, 75015 Paris, France

2Department of Pharmacology and Physiology, University of Rochester, Rochester, NY 14526, USA

3Reference Center for Cerebellar Malformations and Congenital Diseases, Department of Genetics, Trousseau Hospital, AP-HP, 75012 Paris

4INSERM U1141, DHU PROTECT, Robert Debré Hospital, 75019 Paris, France

5Department of Ophthalmology, IHU Necker-Enfants Malades, Paris Descartes University, 75015 Paris, France

6Epithelial biology and disease – Liliane Bettencourt Chair of Developmental Biology, INSERM UMR1163, Imagine – Institute of Genetic Diseases, Paris Descartes University, 75015 Paris, France

7Department of Genetics, IHU Necker-Enfants Malades, University Paris Descartes, 75015 Paris, France

8Department of Genetics, GHU Henri Mondor, 94010 Créteil, France

9Department of Neuroradiology, IHU Necker-Enfants Malades, Paris Descartes University, 75015 Paris, France

10Image at Imagine, INSERM UMR1163, Imagine – Institute of Genetic Diseases, Paris Descartes University, 75015 Paris, France

11Maladies Neurodégénératives, Institut du Cerveau et de la Moëlle Epinière, CHU Paris-GH La Pitié Salpêtrière-Charles Foix, Hôpital Pitié-Salpêtrière, 75013 Paris, France

12Department of Medical Genetics, Faculty of Medical Sciences, University of Campinas, CEP 13083-887, Sao Paulo, Brasil

13Department of Neuropediatrics, Hôpital Trousseau, 75012 Paris, France

14Department of Pediatrics, Hôpital Jean Verdier, 93143 Bondy, France

15Pôle de pédiatrie, Centre d'activité de neurologie pédiatrique, CHU d'Amiens - Hôpital Nord, 80054 Amiens, Cedex 1, France

16Embryology and Genetics of Human Malformation, INSERM UMR1163, Imagine – Institute of Genetic Diseases, Paris Descartes University, 75015 Paris, France

17Genomics Platform, INSERM UMR1163, Imagine – Institute of Genetic Diseases, Paris Descartes University, 75015 Paris, France

18Bioinformatics Platform, INSERM UMR1163, Imagine – Institute of Genetic Diseases, Paris Descartes University, 75015 Paris, France

19Laboratory of Hereditary Kidney Diseases, INSERM UMR1163, Imagine – Institute of Genetic Diseases, Paris Descartes University, 75015 Paris, France

20Laboratoire de Génétique Médicale, Institut de Génétique Médicale d'Alsace, INSERM U1112, Fédération de Médecine Translationnelle de Strasbourg (FMTS), Université de Strasbourg, 67085 Strasbourg, France

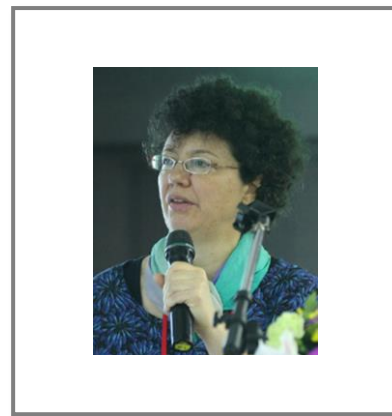
21Service de Génétique Clinique, Hôpital Purpan, 31300 Toulouse, France



## Pr Ginevra Zanni (Italy)

MD, PhD

Expert on the Genetics and Physiopathology of Congenital Ataxias. PhD in Human Genetics and University Assistant in Clinical Genetics at the University Paris. Staff Medical Geneticist at the Department of Neurosciences of the Bambino Gesù Children's Hospital in Rome. Author of several publications on Cerebellar Disorders. Member of the European Reference Network for Rare Neurological Disorders and other international networks for the study of Ataxias.



### **“Identification of novel and hotspot mutations in the channel domain of ITPR1 in two patients with Gillespie syndrome”**

In 1965 F.D Gillespie described a syndrome consisting of cerebellar hypoplasia, mild to moderate intellectual disability and aniridia, characterized by the absence of the pars pupillaris of the iris and the pupillary border. De novo missense mutations in the inositol 1,4,5-triphosphate receptor type 1 gene (*ITPR1*) were reported in more than a dozen of families with Gillespie syndrome (MIM #206700) whereas compound heterozygosity for inactivating mutations in the same gene were identified in a smaller proportion of cases. *ITPR1* encodes an intracellular receptor for inositol 1,4,5-trisphosphate (InsP3) which is highly expressed in the cerebellum and is involved in the regulation of Ca<sup>2+</sup> homeostasis. Missense mutations in the InsP3-binding domain (IRBIT) of *ITPR1* are frequently associated with early onset cerebellar atrophy. In two patients with congenital ataxia, psychomotor delay and iris hypoplasia in which aniridia causing *PAX6* and *FOXC1* were excluded by previous screening, we identified a recurrent deletion (p.Lys2596del) and a novel missense mutation (p.Asn2576Ile) close to a point of constriction in the Ca<sup>2+</sup> pore. Our study expands the mutational spectrum of *ITPR1* and confirms that *ITPR1* screening should be implemented in patients with congenital cerebellar ataxia with or without iris hypoplasia.

## Dr Marta Corton (Spain)

PhD in Biochemistry and Molecular Medicine by University Autonoma of Madrid.

She has 12 years of experience in research of inherited ocular diseases. Since the last 5 years, she is leading an emerging research group focused on the genetic study of eye developmental diseases in the Genetics and Genomics Department of the University Hospital Fundación Jiménez Díaz (Madrid, Spain).



### **“Improving molecular diagnosis of aniridia and WAGR syndrome using customized targeted array-based CGH”**

Congenital aniridia may appear isolated or associated with different systemic anomalies. Up to 30% of cases carry 11p13 deletions showing a high complexity of breakpoints, including partial or whole *PAX6* deletions, microdeletions only affecting 3' *PAX6* enhancers or contiguous gene deletions. The most frequent syndromic form of aniridia, the WAGR syndrome, is a gene contiguous deletion syndrome involving at least *PAX6* and *WT1*. In newborn diagnosed of congenital aniridia, genetic testing is crucial to assess the likelihood of developing in the early childhood the characteristic Wilms tumor of the WAGR syndrome.

Last years, our group have developed different strategies to improve CNV detection in aniridia, WAGR and other anterior segment dysgenesis, using cutting-edge genomic technologies. In 2016, we reported the design of a specific customized high-resolution CGH array, so-called WAGR array, that specifically covers 5 Mb of the WAGR *locus*, and its validation in a large cohort of Spanish patients with aniridia and/or related pathologies. This CGH-array has been updated to include 150 *loci* for eye developmental disorders. We also explored the utility of the next-generation sequencing (NGS) to assess CNVs in the WAGR *locus* by means of targeted panels, including also *PAX6* enhancers and up to 260 genes associated with eye development.

We demonstrated the diagnostic potential of these genomic tools in the genetic testing of patients with aniridia and aniridia-like phenotypes. Their use increased the detection rate of CNVs in our cohort compared to other techniques, resulting in a more complete picture of the genomic *PAX6* deletions. Our approaches allowed not only a more comprehensive and robust analysis for non-coding regulatory *PAX6* regions but also for other rare genes such as *FOXC1* and *PITX2*.

## Pr Eduardo D. Silva (Portugal)

MD, PhD, FEBO

Pediatric ophthalmologist with a PhD in molecular genetics of eye development. Trained at Coimbra University Hospital and Wilmer Eye Institute, Johns Hopkins University, Baltimore MD. Currently working in Centro Cirúrgico de Coimbra, and Hospital Lusíadas in Lisbon. CMA for Coimbra Genomics. Invited Professor at UNIFESP, São Paulo, Brasil and University of Valencia, Spain – post-graduate programs.



### “Management of Axenfeld-Rieger with aniridia”

Axenfeld-Rieger syndrome (ARS) is considered part of the anterior segment dysgenesis spectrum. This term captures the entire phenotypic spectrum continuum. The age of ARS diagnosis varies from birth to adulthood. Appropriate management involves the identification of ocular characteristics and its implications, the identification of associated systemic problems and its potential impact in the patient’s life and surgical management.

Ocular management encompasses appropriate refraction, identification and correction of refractive errors, potential strabismus and amblyopia, addressing important issues like glare and photophobia, and careful monitoring secondary early-onset glaucoma.

Besides regular and timely clinical assessment, structural and functional evaluation allow early diagnosis and medical/surgical decision-making process.

Prescription of glasses, special UV-blocking filters, tinted lenses and, when necessary, low-vision aids allow maximizing visual potential.

The management of glaucoma in ARS is similar to that of other pediatric glaucomas. Medical management is often the preferred therapy for disease onset in late childhood or adolescence, with aqueous suppressants generally being more effective than miotics. In infancy-onset disease, trabeculotomy is favoured over goniotomy. Electrosurgical *ab interno* trabeculotomy (*Trabectome*) techniques may harbour potential. Mitomycin C-augmented trabeculectomy may be a reasonable option in eyes that have failed angle surgery; same applies to Baerveldt Glaucoma Implants (BGI) in combination with XEN gel stents.

The management of ARS require a multidisciplinary approach. Ocular as well as extraocular abnormalities should be recognized in establishing a correct diagnosis, and therapy should address a systematic surveillance of ocular and other developmental abnormalities.

## Pr Ken Nischal (USA)

Prof. Ken Nischal is Chief of the Division of Pediatric Ophthalmology and Strabismus at Children's Hospital of Pittsburgh of UPMC, director of Pediatric Program Development at the UPMC Eye Center, and Professor of Ophthalmology at the University of Pittsburgh School of Medicine.



His focus is on evidence based protocol led clinical care with clinical outcome measures as a source of clinical research. His main areas of clinical research are Anterior Segment Developmental Anomalies Affecting the Cornea, Lens and Trabecular Meshwork. He has published widely in Pediatric Cataract, Glaucoma and Cornea and Craniofacial Anomalies. He has developed an ocular genetic service at Children's Hospital of Pittsburgh and is Co-founder of WSPOS.

### « Management of Peters Anomaly with aniridia »

Peters Anomaly is a term that describes a sign and not a diagnosis. It is a condition where there is a corneal opacity obscuring the visual access either completely or partially. Aniridia is the absence either partial or complete of the iris. The most common form of Aniridia is due to PAX6 mutations or deletions and in this condition, occasionally the lens can be attached to the cornea and give a corneal opacification that can be termed Peters Anomaly Type II. However, there are other conditions where the iris does not form and where corneal opacification is more prevalent. One of these conditions is Axenfeld-Rieger syndrome due to a FOXC1 or PITX2 deletion or mutation, which can result in corneal opacification consistent with Peters Anomaly and partial or complete Aniridia.

There are other instances where the Iris is missing either because it never formed or it formed and became atrophic during the child's development in the mother's uterus. In either case, the bottom line is that the child will have severe visual deprivation if the corneal opacity is not dealt with or if the amount of light entering the eye is not increased

In cases of PAX6 related aniridia with keratolenticular adhesion (lens attached to the cornea) causing the corneal opacity, rather than doing the corneal transplant, it is sometimes easier to remove the lens; which often has a cataract in it and correct the refractive error with a contact lens. This often results in functional vision and this method of treatment has been published previously. In cases where a corneal transplant has to be done because the cornea has a large opacity in it, in the absence of iris there are techniques that have to be engaged to make sure that the lens stays in place and it does not have to be removed. These techniques involve distinct techniques of pediatric corneal transplantation and involve meticulous surgical techniques.

Whenever a child has such complex anterior segment eye problems, a full genetic and pediatric evaluation is essential to make sure that the underlying diagnosis is sought and not overlooked.

# **SESSION 4 :** **POSTERS**

## **POSTER 1**

### **“Outcomes of allogenic cultivated limbal epithelial stem cells in aniridia patients suffering from severe limbal stem cell deficiency”**

**Behaegel** J1,2,3,4, Koppen C2,3, Leysen I2, Vanthillo T1, Tassignon MJ2,3, Zakaria N1,3, Ni Dhubhghaill S2

1 Centre for Cell Therapy and Regenerative Medicine UZA, Antwerp, Belgium

2 Dept of Ophthalmology, Antwerp University Hospital, Antwerp, Belgium

3 Faculty of Medicine and Health Sciences, Department of Ophthalmology, Visual Optics and Visual Rehabilitation, Antwerp University, Antwerp, Wilrijk, Belgium

4 Faculty of Medicine and Pharmacy, Vrije Universiteit Brussel, Brussels, Belgium

#### **Introduction**

Aniridia-related keratopathy (AAK) is one of the ocular abnormalities of congenital aniridia. It occurs as a result of peripheral and progressive conjunctivalization of the corneal surface, which is caused mainly by limbal stem cell deficiency (LSCD). These abnormalities develop later during life and form a significant threat to vision. Cultivated limbal stem cell transplantation (CLET) is one of the treatment options for patients with severe LSCD. The grafts aim to restore the limbal barrier and reconstruct the ocular surface, improving rates of subsequent corneal graft survival.

#### **State of the question**

Do allogeneic limbal epithelial stem cell grafts improve the ocular surface in aniridia patients suffering from severe LSCD?

#### **Purpose**

To assess the results of transplanting allogenic, non-xenogenic, bioengineered, composite grafts of cultured limbal epithelial cells on standardized amniotic membranes in aniridia patients suffering from LSCD.

#### **Methodology**

Six patients with aniridia and a total limbal stem cell deficiency have received an allogenic stem cell grafts between January 2010 and March 2017. The limbo-amnion grafts were generated by cultivating limbal epithelial stem cells from an HLA-matched living related (n=5) or cadaveric (n=1) donor eye on a standardized amniotic membrane. The cells were cultured for a period of 2 weeks in a xenogenic-free culture medium and the composite graft was transplanted with a standardized 'no touch' surgical technique.

#### **Envisaged sequence of research: /**

#### **Results**

One patient was excluded because of unrelated side effects. The mean follow-up of the remaining patients is 41,2 months (range 6 – 82 months). An anatomical success was noted in two eyes, meaning a stable ocular surface and a reduction of the corneal neovessels. One eye had a mild recurrence of the corneal vessels and therefore was graded as 'partial success'.

The two remaining patients had initially a stable ocular surface, but their results failed on the long-term (follow-up of more than 2 and 6 years respectively).

#### **Conclusions/Expected consequences**

Transplantation of allogenic ex vivo cultivated limbal stem cells in aniridia patients may provide improvement in ocular surface stability because it temporarily restores the limbal microenvironment and the anterior cornea. However, it does not appear to be a long-term solution for visual improvement in this small study population. A longer follow-up period and inclusion of more patients are needed to confirm these findings.

## **POSTER 2**

### **“Spectral--Domain Optical Coherence Tomography findings in 64 patients with aniridia : a retrospective study”**

**Alejandra Daruich**, Matthieu Robert, Zoia Mincheva, Nathalie De Vergnes, Dominique Bremond-Gignac, Service d'Ophtalmologie, Hôpital Necker-Enfants malades, Paris, France

#### **Introduction**

Foveal hypoplasia has been described in patients with aniridia and PAX--6 mutation. Spectral-domain optical coherence tomography (SD--OCT) has improved the evaluation of foveal architecture. An SD--OCT grading system of foveal hypoplasia of different etiologies, has been recently proposed indicating a correlation with visual acuity.

#### **State of the question**

Foveal hypoplasia has been so far poorly characterized in aniridia patients. Correlation with PAX-6 mutation and impact on visual acuity should be further investigated.

#### **Purpose**

To evaluate foveal anomalies on SD--OCT in aniridia patients.

#### **Methodology**

Consecutive patients presenting with aniridia between September 2015 and April 2018 were retrospectively reviewed. Clinical and genetic characteristics and SD--OCT finding were analyzed. Foveal hypoplasia was classified according to Thomas et al. as grade 1 to 4. The Spearman coefficient was used to assess correlations.

#### **Results and expected consequences**

Charts of 145 patients with aniridia were reviewed.

SD--OCT could not be performed in 81 patients because of young age, corneal or lens opacification.

Sixty--four aniridia patients (36 females and 28 males) with SD--OCT records were included. Mean age was  $22.3 \pm 20.3$  years. 91% of patients presented with isolated aniridia and 9% with syndromic aniridia. PAX6 mutation was confirmed in 47% of patients.

SD--OCT was performed bilaterally in 49 patients and unilaterally in 15 patients.

A total of 113 eyes were analyzed. The majority of eyes showed grade 4 hypoplasia (n=90, 79.6%). Grade 3 hypoplasia was found in 2 eyes (1.8%), Grade 2 in 8 eyes (7.1%) and grade 1 in 8 eyes (7.1%). Three patients (n=5, 4.4%) presented with normal foveal characteristics on SD--OCT. LogMar BCVA was significantly correlated with the degree of foveal hypoplasia on SD--OCT ( $p < 0.001$ ,  $r = 0.5$ ). All patients with PAX--6 mutation showed grade 4 foveal hypoplasia.

**In conclusion**, the majority of aniridia patients presented severe foveal hypoplasia on SD--OCT, that was associated with lower visual acuity SD--OCT could help clinicians to predict visual prognosis in patients with aniridia. Genetic alterations such as PAX6 mutations may explain the co-existence of foveal and iris developmental defects.

## **POSTER 3**

### **“Towards Allele-Specific CRISPR Gene Editing to Prevent Vision Loss in a Novel Mouse Model of Aniridia”**

**Zeinab Mirjalili Mohanna, Siu Ling Lam, Tom W. Johnson, Tess C. Lengyell, Elizabeth M. Simpson.**

The paired box 6 gene (PAX6) encodes a conserved transcription factor that controls many aspects of early development of the central nervous system, eye, and some non-neuronal tissues such as the pituitary and pancreas. Heterozygous loss-of-function mutations of *PAX6* result in a rare congenital disorder, known as aniridia. Aniridia is a syndrome, but is best known for the iris hypoplasia visible in the child’s eyes at birth. Currently, there is no cure or long-term therapy for aniridia. One possible approach to treating aniridia is gene-editing therapy.

We hypothesize that CRISPR-mediated gene editing can increase the expression of PAX6 protein, improve the function of the neural and other tissues of the eye, and ultimately rescue the mutant phenotype. Towards this end, we carried out cell-based optimization of guide RNAs (gRNAs) in *Pax6* mouse embryonic stem cell cultures. Purified SpCas9, template DNA, and several synthetic candidate gRNAs were delivered to the cells by electroporation. Functionality of each gRNA was first validated by Sanger sequencing, and then quantified by site-specific next generation sequencing. Excitingly, compared to the control, our best gRNA corrected the *Pax6* mutation in 15% of the cells. Furthermore, initial studies show no mutagenesis of the wild-type allele. If off-target becomes a concern, an alternative approach would be to replace SpCas9 with either eSpCas9 or SpCas9-HF1, which have been shown to confer the same on-target activity, but considerably reduced off-target activity.

To assess accurately the efficiency of our gene therapy, it is important to be able to distinguish wild-type from corrected PAX6. However, in the current *Pax6 Sey* mouse model the two proteins would be identical. To circumvent this problem, we created a new mouse model of aniridia using CRISPR technology to add a 3xFLAG “tag” on the *Sey Pax6* gene. Such a tag will allow antibodies to distinguish the wild-type and CRISPR-corrected PAX6 proteins. Our next objective is to use our optimized correction assay, and new tagged-mouse model to correct the *Pax6-Sey* mutation *in vivo* using a dual recombinant Adeno-associated viral system.

We expect in CRISPR/Cas9 treated mice to detect 3xFLAG PAX6 by western blot analysis of “corrected” tissues, and observe improved function of the retina, normalization of the thickness of the cornea, and improved electroretinogram wave structure compared to untreated controls.



## **POSTER 4**

### **“Within-family phenotype-genotype discordance in congenital aniridia”**

**Hilde R. Pedersen**<sup>1</sup>, Maureen Neitz<sup>2</sup>, Stuart J. Gilson<sup>1</sup>, Erlend C. S. Landsend<sup>3</sup>, Øygunn A. Utheim<sup>3</sup>, Tor P. Utheim<sup>1,3</sup> and Rigmor C. Baraas<sup>1</sup>

<sup>1</sup>National Centre for Optics, Vision and Eye Care, Faculty of Health and Social Sciences, University of South-Eastern Norway, Kongsberg, Norway

<sup>2</sup>Department of Ophthalmology, University of Washington, Seattle, WA, USA

<sup>3</sup>Department of Ophthalmology, Oslo University Hospital, Oslo, Norway

#### **Purpose**

Congenital aniridia is an autosomal dominant disorder affecting retinal development and is associated with varying degrees foveal hypoplasia. We investigated photoreceptor structure in a family with aniridia caused by a deletion in the *PAX6* gene to increase the understanding of the complexity and variation in retinal phenotype between the individuals.

#### **Methods**

Seven persons (4 males) with congenital aniridia, aged 24–66yrs, and 30 age-matched normal controls (13 males), including 4 unaffected family members, were included in the study. DNA was isolated from saliva samples and used in the polymerase chain reaction (PCR) to amplify and sequence the exons and intron/exon junctions of the *PAX6* gene. Fluorescent DNA sequencing was performed on both DNA strands. Visual acuity and ocular media opacities were evaluated. High-resolution retinal images of the macular region were acquired with Heidelberg Spectralis SD-OCT2 and adaptive optics scanning light ophthalmoscopy (AOSLO). Individual cone photoreceptors were identified and cone density (CD; cones/mm<sup>2</sup>) was estimated from foveal center to 5° eccentricity along the nasal-temporal meridians. Horizontal SD-OCT line scans were segmented to analyze severity of foveal hypoplasia (normal to complete: 0–4).

#### **Results**

DNA sequencing revealed a deletion of the -2 nucleotide in intron 2 (IV2-2delA), disrupting the consensus 3' splice site for exon 3; a known aniridia-causing mutation. Those with aniridia had variable iris phenotype ranging from almost normal appearance to absence of the iris. Visual acuity ranged from 0.20–0.86 logMAR. Four with aniridia had foveal hypoplasia grade 2 (all males) and three had grade 3 (all females), two of the females also had optic nerve hypoplasia and nystagmus. AOSLO images were obtained of five (4 males) family members with aniridia and showed variable cone packing. Foveal CD varied between 17831 and 53683 cones/mm<sup>2</sup> with overlap between foveal hypoplasia grade 2 and 3. There was no obvious relationship between foveal CD and visual acuity. CD was significantly lower in aniridia, with CD >3 SD below the normal mean within 0.5°, >2 SD below the normal mean at 0.5°–4°, and >1SD below the normal mean at 5° eccentricity.

#### **Conclusions**

The results show considerable difference in structural phenotype in family members with aniridia and the same genetic mutation; females being more affected than males. The weak of relationship between CD and visual acuity suggests that visual function in aniridia also may be related to altered numbers of post-receptoral neurons and development of the associated circuitry.

## **POSTER 5**

### **“Colour Vision in Aniridia”**

**Rigmor C. Baraas**<sup>1</sup>, Hilde R. Pedersen<sup>1</sup>, Lene A. Hagen<sup>1</sup>, Erlend C. S. Landsend<sup>2</sup>,

Øygunn Aa. Utheim<sup>2,3</sup>, Tor P. Utheim<sup>1,2,3</sup>, Stuart J. Gilson<sup>1</sup> and Maureen Neitz<sup>4</sup>

<sup>1</sup>National Centre for Optics, Vision and Eye Care, Faculty of Health and Social Sciences, University of South-Eastern Norway, Kongsberg, Norway

<sup>2</sup>Department of Ophthalmology, Oslo University Hospital, Oslo, Norway

<sup>3</sup>Department of Medical Biochemistry, Oslo University Hospital, Oslo, Norway

<sup>4</sup>Department of Ophthalmology, University of Washington, Seattle, WA, USA

#### **Purpose**

Aniridia is a congenital disorder typically caused by a PAX6 gene mutation associated with iris and foveal hypoplasia and nystagmus. Severe phenotypes are also associated with secondary pathology such as keratopathy, cataract, and glaucoma. Recently, we assessed colour vision and its association with retinal development in congenital aniridia (Pedersen et al., 2018).

These results are of importance, not only for clinicians in facilitating diagnosis and prognosis, but also for advancing the understanding about timing and degree of foveal formation disruption with regards to phenotypic severity.

#### **Methods**

Thirty-six persons with congenital aniridia (10–66 yrs), and 52 healthy normal trichromatic controls (10–74 yrs) participated in the study. Colour vision was examined binocularly with HRR pseudoisochromatic plates (4th ed., 2002) and a low vision version of the Colour Assessment and Diagnosis (CAD) test. Genetic testing was performed to identify or confirm the genetic cause of aniridia in each participant. Cone-opsin genes were analysed to confirm that none of the participants had congenital colour vision deficiencies. The CAD stimuli were double its default size to compensate for reduced visual acuity in aniridia. Luminance noise was increased, and temporal frequency lowered to mask rod intrusion. Chromatic sensitivity was measured along 16 hue directions and mean red-green (RG) and yellow-blue (YB) thresholds were computed. Visual acuity and ocular media opacities were assessed. The central 30 degrees of both eyes were imaged with the Heidelberg Spectralis OCT to grade the severity of foveal hypoplasia (FH, normal to complete: 0–4).

#### **Results**

Eleven (35.5%) with aniridia made two or more RG errors on HRR, four (12.9%) of whom also made YB errors; while one made YB errors only. In aniridia, 19 participants had higher CAD RG thresholds of which eight also had higher CAD YB thresholds, than normal controls. Individuals with aniridia had a quantifiable loss of colour vision with greatest loss in red-green colour discrimination. Aniridia patients with complete FH had significantly higher RG thresholds than those with mild FH ( $P=0.038$ ). Additional increase in YB threshold was associated with secondary ocular pathology.

#### **Conclusion**

Visual function loss in aniridia is not limited to loss of visual acuity. Additional loss of color vision appears to be a combined consequence of the timing of arrested foveal formation, associated alterations in retinal structure and processing and secondary ocular pathology.

#### **References**

Pedersen HR, Hagen LA, Landsend ECS, et al. Color Vision in Aniridia. *Investigative Ophthalmology & Visual Science*. 2018;59:2142-2152

## **POSTER 6**

### **“Activity of *Pax6*<sup>+/-</sup> limbal epithelial stem cells in vivo”**

**J Martin Collinson**

School of Medicine, Medical Sciences and Nutrition, University of Aberdeen

Corneal inflammation and opacity in aniridia (aniridic keratitis, also called aniridia-related keratitis or ARK), presents with symptoms similar to known limbal stem cell deficiencies, and is usually treated as such.

However our previous work using chimeric mice has shown that *Pax6*<sup>+/-</sup> limbal stem cells can in some circumstances populate and maintain healthy sectors of the corneal surface.

The objective of this study was to directly assay the activity of *Pax6*<sup>+/-</sup> limbal epithelial stem cells in vivo and to determine whether they can respond to corneal wounding.

A label-retaining assay was used to identify limbal epithelial stem cells. Adult *Pax6*<sup>+/-</sup> mice and their wild-type littermates were given the thymidine analogue, iododeoxyuridine (IdU) for 28 days, followed by 9 weeks of washout. Label retaining cells were detected in *Pax6*<sup>+/-</sup> limbal epithelia, consistent with survival of slow-cycling stem cells in aniridia. Double-labelling with EdU at the end of the washout period allowed identification and quantification of actively proliferating stem cells.

The proportion of actively dividing stem cells was compared between wild-type and *Pax6*<sup>+/-</sup> mice, and also after corneal wounding in vivo.

It is concluded that *Pax6*<sup>+/-</sup> mice have active, functional limbal epithelial stem cells. The ocular surface abnormalities associated with aniridia may be due to the regenerative potential of the stem cells being overwhelmed by fragility and accelerated corneal epithelial turnover in aniridic eyes. It suggests that effective therapy for ARK should not only target the stem cells but also take account of corneal epithelial cellular health.

## **POSTER 7**

### **“Genetic abnormalities in Bulgarian patients with aniridia”**

**Maya Atanasoska**<sup>1</sup>, Veselin Kukenski<sup>2</sup>, Radoslava Vazharova<sup>1, 2</sup>, Lubomir Balabanski<sup>1</sup>, Samuil Ivanov<sup>1</sup>, Anna Popova<sup>3</sup>, Silvia Andonova<sup>4</sup>, Alexey Savov<sup>4</sup> and Draga Toncheva<sup>1, 5</sup>  
1Gynecology and Assisted Reproduction Hospital „Malinov MD“, Genetic laboratory, Sofia, Bulgaria; 2Sofia University St. Kliment Ohridski, Faculty of Medicine, Department of Biology, Medical genetics and Microbiology, Sofia, Bulgaria; 3 Medical Faculty, Diagnostic and Consulting Center, Pediatric Ophthalmology, University Alexandrovska Hospital, Sofia, Bulgaria, 4National Genetic Laboratory, UHOG „Maichin dom“, Medical University of Sofia, Sofia, Bulgaria; 5 Department of Medical Genetics, Medical University of Sofia, Sofia, Bulgaria

Introduction

Aniridia is a rare genetic disorder characterized by a variable degree of iris hypoplasia. Several others ocular features present from the birth or arise progressively through time. The worldwide incidence of this condition is between 1/40 000 and 1/ 100 000. About 2/3 of the cases are familial with autosomal-dominant inheritance; the remaining 1/3 are sporadic as result of de novo mutation. To the best of our knowledge there are 14 registered cases of aniridia in Bulgaria; half of them are children under the age of 12 years.

#### **The aims of our project were**

- (1) To analyze the genomic abnormalities of the available Bulgarian patients with Aniridia;
- (2) To determine microstructural aberration associated with the disease;
- (3) To identify pathological variations in *PAX6* gene.

#### **Materials and Methods**

A total of 9 patients (5 females and 4 males) with a clinical diagnosis of Aniridia were studied to reveal the genetic basis of the condition. No patients exhibit aniridia as part of a syndrome. Three of the studied patients were part of one family with clear dominant mode of inheritance. A SNP array and Sanger Sequencing analysis were performed in order to confirm the aniridia diagnosis. The sequencing was performed with a custom-made set of primers, covering all the exons and exon-intron boundaries of the gene *PAX6*.

#### **Results**

The conducted assays uncovered a potential molecular cause for 5 out of 9 patients - 2 full gene deletions, a small scale duplication, a single base deletion and a splice site disrupting SNP were detected. The SNP array identified two pathological heterozygous deletions (11p13 and 11p14.1p13), both of them covering the *PAX6* and *ELP4* genes. Sanger sequencing detected potential pathological variants in the *PAX6* gene in three patients, all sporadic cases.

#### **Conclusion**

Our study demonstrated the heterogeneous nature of Aniridia and its variable clinical feature. Approximately 75% of registered cases in the Bulgarian population were studied; 5 unique disease-related variants were discovered. Two out of 3 variants revealed by Sanger sequencing have not been previously reported. This is the first study in Bulgaria which applies genetic analyses like genome-wide SNP array and Sanger sequencing on Aniridia patients.

## **POSTER 8**

### **“Phenotypic study of PAX6 haploinsufficient aniridic corneal stromal cells in a corneal tissue equivalent”**

**Carla Sanchez-Martinez**<sup>1</sup>, Victoria Tovell<sup>1</sup>, Stephen Tuft<sup>2</sup>, Julie T Daniels<sup>1</sup>

1. UCL, Institute of Ophthalmology London, UK

2. Moorfields Eye Hospital NHS Foundation Trust, London, UK

Aniridia is a congenital eye disorder caused by a haploinsufficiency of the PAX6 transcription factor, an essential transcription factor for the correct development of the eye during the embryonic stage. Aniridic patients present opacification and vascularisation of the cornea, a condition known as aniridia related keratopathy (ARK). This is a progressive manifestation that can lead to sight loss. ARK represents an unmet clinical need, as treatments are not always available or successful but to develop new efficient therapies, it is essential to better understand the biological mechanisms behind ARK.

Most research studies focus on the corneal epithelium, attributing ARK to a limbal epithelial stem cell deficiency.

This project is based on the hypothesis that the aniridic corneal stromal cells are also affected and contribute to the development of ARK.

For the first time, we cultured and studied the phenotype of human aniridic corneal stromal cells (ACSC) and control corneal stromal cells (CCSC) inside a corneal tissue equivalent (TE) to identify any ARK specific changes. ACSC were isolated from fresh corneas (N=3) and genotyped to identify their *PAX6* variants. CCSC were isolated from organ-cultured corneas (N=3) and both ACSC and CCSC were cultured inside a compressed collagen type I gel for 21 days.

Supernatant was collected at day 7, 14 and 21 for assessment of Matrix metalloproteinases (MMP) concentration by Zymogram and ELISA, as an indication of the cell capacity to remodel the extracellular matrix.

At the end point, one third of the gels were fixed, stained and morphology was recorded. Alignment of the cells was also calculated with Fiji ImageJ 1.49s software. Another third of the gels were used for life/dead analysis as a measure of apoptosis. The remaining gels were used for RNA isolation and subsequent qPCR analysis. Two-way repeated-measures ANOVA and two-tailed Student's t-test (Image analysis) were used for the statistical analysis. ACSC and CCSC were successfully isolated, cultured and expanded. One run-on (NM\_000280: p.\*423Leuext\*?) and two premature stops (NM\_000280: p.(Gly194\*), NM\_000280: p.(Arg125Serfs\*7)) variants were identified in the *PAX6* gene of the three aniridic samples, respectively.

When cultured inside the TE, ACSC secreted lower amounts of MMP-1 and MMP-2 than CCSC at day 14 ( $p < 0.05$ ) and 21 ( $p < 0.001$ ) (ELISA), in accordance with zymography and qPCR results. Cell distribution and morphology also differed between ACSC and CCSC. While CCSC appeared to be thin and elongated (Circularity=0.135), ACSC had a wider range of morphologies with many cells presenting a rounded body with protrusions (Circularity= 0.321,  $p < 0.001$ ). CCSC presented a 75% of alignment with each other, whereas ACSC showed only 39%.

Interestingly, no differences were found in apoptosis between ACSC and CCSC in these cultured conditions.

In the native cornea, stromal cells are quiescent and align inside the collagen matrix.

Evident differences between the two cells phenotypes in culture indicate that the ability of ACSC to remodel the matrix might be impaired and this might contribute to the development of ARK.

## **POSTER 9**

### **“Functional splicing assays using *in vitro* minigenes to assess variants of uncertain significance in PAX6”**

**María Tarilonte Misas**, I am PhD student in Lab. Genetics and Genomics in Biomedical Research Institute Fundación Jiménez Díaz University Hospital (IIS-FJD, UAM).

#### **INTRODUCTION**

*PAX6* encodes a highly conserved transcription factor that plays pivotal roles in normal ocular development. Dominant *PAX6* defects can result in a spectrum of ocular developmental disorders, ranging from panocular forms of aniridia to mild forms of Peter's anomaly, ocular coloboma and isolated foveal hypoplasia, depending on mutation type and gene dosage. Aniridia, characterized by complete or partial absence of the iris and foveal hypoplasia, represent the most frequent *PAX6*-related condition. Most of aniridia patients carry loss-of-function variants leading to premature termination codon and then, are expected to cause *PAX6* haploinsufficiency. However, the disease mechanisms are not well known for other rare *PAX6* variants of uncertain significance (VUS) and then, its pathogenicity could not be straightforward addressed.

#### **STATE OF QUESTION**

Emerging *PAX6* gene screening by next generation sequencing (NGS) identifies non-coding and coding VUS in a significant proportion of patients with *PAX6*-associated phenotypes. Functional and clinical interpretations of these VUS represent a real challenge for genetic counseling of patients and families.

#### **PURPOSE**

In view of the increasing implication of genetic variants in pathological processes underlying splicing disruption, we aimed to assess the pathogenicity of several VUS in *PAX6* using functional splicing analysis by *in vitro* minigene assays.

#### **METHODS**

Targeted NGS for the *PAX6* locus was used to screen a cohort of 47 Spanish and French patients, in which pathogenic coding variants were not previously identified. Different experimental approaches were performed to assess the impact of different VUS on splicing. *In vitro* assays were carried out using *PAX6* minigenes for different exons, in which mutant alleles were introduced by site-directed mutagenesis. *In vivo* RT-PCR expression analysis was also performed in lymphocytes cells lines (LCLs) from patients.

#### **SEQUENCE OF RESEARCH**

We studied several VUS, not causing a priori *PAX6* haploinsufficiency, such as non-coding 5'UTR and intronic variants, and also coding variants (missense, in-frame indels or splicing-site variants). First, splicing effect of these variants were assessed using several *in silico* tools. Further, *in vitro* minigene assays and *in vivo* expression studies if LCLs were available, allowed us to analyze the functional consequences on canonical splicing.

#### **RESULTS AND CONSEQUENCES**

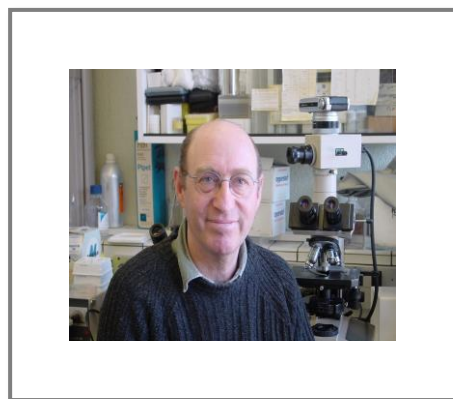
Our work confirmed the putative *in silico*-inferred spliceogenic effect for several VUS variants, demonstrating accurately splicing disruption. Thus, *PAX6* minigenes represent an accurate alternative to analyze the functional consequences of VUS on splicing. In consequence, the clinical interpretation of several genetic variants found in our cohort, that were initially classified as VUS or even "likely benign", was reassessed and were finally considered as disease-causing variants.

**SESSION 5 :**  
**ANIRIDIA,**  
**OCULAR SURFACE**  
**AND CORNEA**  
**REHABILITATION**

## Pr Daniel Aberdam (France)

PhD

Prof. Daniel Aberdam completed his first degrees in life sciences at Pierre and Marie Curie University (Paris, France) and his PhD at the Weizmann Institute (Israel) on the oncogenic potential of homeotic genes. During his postdoctoral stage, he discovered the identity of genes responsible for severe human skin diseases that then allowed the first antenatal diagnosis for junctional epidermolysis bullosa syndromes. Then, his scientific interests turned on epidermal gene regulation with the identification of skin specific promoters. Then, his group has focused on the physiopathology of stem cells and designed original cellular models from embryonic stem cells that recapitulate embryonic normal and pathological skin and cornea formation. It allows the characterization of genes and signaling pathways involved in these critical steps and their involvement in skin defects found in patients affected by human congenital disorders, like ectodermal dysplasia syndromes. This team was the first to demonstrate the ability of pluripotent stem cells to produce a full thickness skin and to use unusual metabolic pathway for pluripotency. More recently, the group used cell reprogramming of patient samples modeling in vitro genodermatoses and identified small compounds able to rescue the phenotype.



### **“In vitro modeling of aniridia-related keratopathy (ARK). Potential for drug discovery and therapy”**

Lauriane Roux<sup>1</sup>, Isabelle Petit<sup>1</sup>, Jean-Paul Concordet<sup>2</sup>, Olivier Ferrigno<sup>3</sup> and Daniel Aberdam<sup>1</sup>

1INSERM U976, Hôpital St-Louis, Paris, France ,

2CNRS UMR 7196 / INSERM U1154, Muséum National d'Histoire Naturelle, Paris, France,

3INSERM U938, Centre de Recherche Saint-Antoine, Paris, France

Haploinsufficiency of PAX6 in humans is the main cause of congenital aniridia, a rare eye disease characterized by iris hypoplasia and reduced visual acuity. Patients have also progressive disorders including cataract, glaucoma and corneal abnormalities making their condition very challenging to manage. Aniridia-related keratopathy (ARK), caused by a combination of factors including limbal stem-cell deficiency, impaired healing response, abnormal differentiation, and infiltration of conjunctival cells onto the corneal surface, affects up to 95% of patients. It usually begins in the first decade of life resulting in recurrent corneal erosions, sub-epithelial fibrosis with corneal decompensation and opacification.

Unfortunately, current treatment options for aniridia patients are currently limited. Although animal models partially recapitulate this disease, there is no in vitro cellular model of AKT needed for drug/therapeutic tools screening and validation.

We used genome editing (CRISPR/Cas9 technology) to introduce a nonsense mutation into one allele of the PAX6 gene in limbal cells, a reservoir of stem cells able to differentiate into corneal cells upon calcium raise at confluency. A resulting mutated clone, expressing half of the amount of PAX6 protein and thus representative of haploinsufficiency, was further characterized.

Sequencing analysis showed that no off-target mutations were induced. The mutated cells displayed reduced cell proliferation and cell migration but enhanced cell adhesion. Known PAX6 targets expression was also reduced. Remarkably, addition of recombinant PAX6 protein was able to activate endogenous PAX6 gene and, as a consequence, rescue the phenotype.

Our unique in vitro model recapitulates well the human disease, suggests recPax6 as a therapeutic tool and is powerful to identify drugs that could rescue the corneal defect but also lens and retina defects related to aniridia.

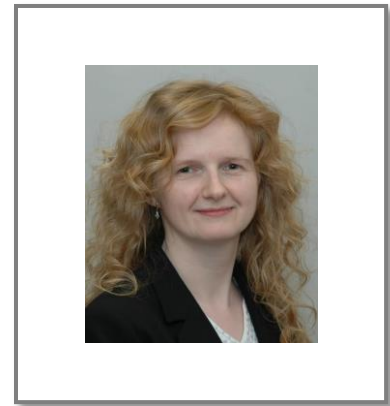


## **Pr Julie T. Daniels (UK)**

PhD

Professor of Regenerative Medicine and Cellular Therapy, UCL  
Institute of Ophthalmology

Director of the Cells for Sight Advanced Therapy Manufacturing  
Unit, Moorfields Eye Hospital, London UK



### **“Biology and future therapeutic possibilities for aniridia-related keratopathy”**

Aniridia is an orphan heritable disease (1:100,000 incidence) in which PAX6 gene mutations can cause severe and sight threatening developmental eye problems. Aniridia-related keratopathy (ARK) manifests as persistent, chronically painful defects of the outer epithelial layer of the cornea, vascularisation and scarring. This correlates with dysfunction in the ability of limbal epithelial stem cells (LESC), stromal cells and matrix to maintain normal corneal epithelium. ARK induced light sensitivity can lead to social exclusion. Current treatments include whole tissue transplantation and cultured LESK therapy which have both shown poor long-term outcomes. This may in part be due to 1) antigen presenting cell presence in whole tissue transplantation and 2) stromal cell absence in cultured LESK therapy. An optimal ARK treatment would include transplantation of both healthy LESK and a stroma populated with stromal cells. This presentation will present a proposed solution, and its rationale, which utilises our patented technology known as RAFT (Real Architecture for 3D Tissues) in which LESK and stromal cells are co-cultured in a transplantable type I collagen-based tissue equivalent. Good manufacturing practise (GMP) protocols have been established in our pre-GMP laboratories and RAFT has undergone pre-clinical safety studies in a rabbit model. Having obtained regulatory advice (MHRA) we will now, subject to funding, work towards achieving 1) GMP protocol validation in our Cells for Sight MHRA/HTA licensed manufacturing facility, 2) submission of the required documents for clinical trial authorisation and 3) proceed towards a first in human RAFT transplantation study in patients with ARK.

## **Pr Eric Gabison (France)**

MD, PhD

Eric E. Gabison is Professor of Ophthalmology, co-chair of the Ophthalmology department. Head of the cornea and external disease department and director of the Translational Research and Experimental Corneal Surgery Laboratory at the Rothschild Ophthalmology Foundation. He is currently conducting a national cell therapy clinical trial for the treatment of corneal chemical burns.



### **“Aniridia and corneal ulcer therapy “**

Corneal involvement in Aniridia combines progressive loss of corneal transparency and recurrent erosion that may lead to corneal ulceration. This Epithelial instability is a common feature in the pathogenesis of corneal epithelial stem cell deficiencies quickens the progressive loss of corneal transparency. The management of Aniridia related keratopathy includes not only the treatment of corneal erosions and ulcerations but also the prevention of their recurrence. Autologous serum, amniotic membrane, inhibition of Matrix Metalloproteinases, scleral contact lenses are some of the therapeutic arsenal commonly used to favor corneal healing.

## **Dr Maria Fideliz de la Paz (Spain)**

MD PhD

Consultant of Anterior Segment Department at the Centro de Oftalmología Barraquer in Barcelona, Spain since 2008. Specialist in ocular surface diseases like dry eye, allergies, chemical burns, autoimmune disease, limbal stem cell disease. Corneal transplant, tumors and keratoprosthesis surgeon. Member of the Steering Committee of the International Keratoprosthesis Study Group.

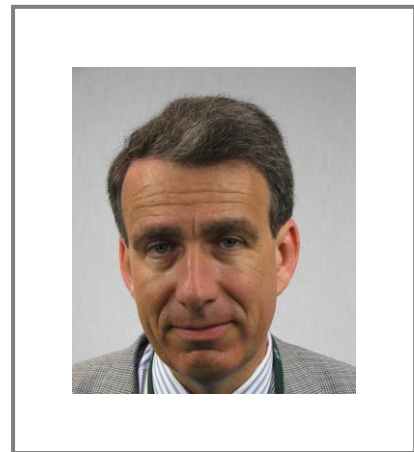


### **”Aniridia and Boston type I keratoprosthesis : anatomical survival and visual prognosis, post-surgical visual outcome and complications”**

The Type 1 Boston Keratoprosthesis is the most widely implanted artificial cornea in the U.S and around the world due to its ease of implantation for any corneal surgeon. However, several complications like glaucoma and retro-prosthetic membrane are serious complications requiring long-term experience for detection and management for the KPro surgeon. International experience on its use in congenital aniridia shows overall initial improvement in visual acuity in the majority of patients. However, progressive loss of visual acuity thru time may occur due to high incidence of retro-prosthetic and retro-backplate membrane formation, and glaucoma onset and progression. Anatomic retention is satisfactory. Videos of tectonic graft in a case of prosthesis extrusion in a case of congenital aniridia will be presented.

## Pr Vincent Borderie (France)

Vincent Borderie is professor of ophthalmology at Sorbonne Université, Paris, France. He is chairman of the department of ophthalmology V at the French National Eye Hospital (CHNO des 15-20) and coordinator of the ophthalmology residency program for Paris region. He has served as president of the European Eye Bank Association and 10th Boston Visiting Professor in Cornea and External Eye Disease (Harvard Medical School, Boston, USA).



### “The limbal stem cell niche”

Vincent M. Borderie, Djida Ghoubay, Cristina Georgeon, Nilly Banayan, Kate Grieve, Institut de la Vision, Centre Hospitalier National d’Ophtalmologie des 15-20, Sorbonne Université, INSERM, CNRS UMR 7210, Paris, France

The limbal stem cell niche is a structure of the ocular surface constituting the anatomic border of the conjunctiva and the central cornea. It is a tank for limbal stem cells (LSC) which renew the corneal epithelium during life. This microenvironment contributes to the development and maintenance of the various unique features that characterize a stem cell niche. It is made up of extracellular matrix components, other resident cells including stromal stem cells (SSC) and melanocytes, and the signals they release. The corneoscleral limbus offers such a distinctive protected environment, which is characterized by dense vascularization, innervation, and protection from potential damage by UV-light by the presence of melanin pigmentation. The region of limbal palisades and crypts and the corneal transition zone appear to be involved in providing unique microenvironments for corneal epithelial stem and progenitor cells, by controlling growth factor signaling. In normal humans, limbal crypts are detected in 90% of cases. Crypts extending between the palisades of Vogt into the cornea are radial or rounded, and often interconnected by circumferential crypts extending beneath the scleral surface. Limbal crypts are more numerous on the vertical axis than on the horizontal axis. They feature presence of p63- $\alpha$ + cells and cytokeratin-3+ cells. Colony Forming Efficiency, a marker of limbal stem cells, increases with limbal crypt volume. Modern imaging technologies (i.e., in vivo confocal microscopy and spectral domain optical coherence tomography) allow in vivo non-invasive and precise assessment of the limbal niche condition. Aniridia-associated keratopathy (AAK) features damages to the limbal niche including loss of the palisades of Vogt.

The two stem cell populations present in the limbus (i.e., LSC and SSC) carry the PAX6 gene mutations in aniridia patients. This may explain why transplantation of allogeneic cultured LSC in AAK, which does not regenerate the limbal niche, usually fails after a few months or years. Development of further technologies aiming at regenerating the limbal niche is needed to improve the prognosis of AAK. Both LSC and SSC can be obtained from small human limbal explants and grown in feeder-free and xeno-free specific culture media. LSC are characterized by colony formation and expression of PAX6,  $\Delta$ NP63 $\alpha$ , Bmi1, ABCG2, SOX9 and vimentin, with a few cells positive for CK3. SSC are characterized by sphere formation, expression of PAX6, SOX2, BMI1, NESTIN, ABCG2, KERATOCAN, VIMENTIN, SOX9, SOX10 and HNK1, production of collagen fibrils and differentiation into keratocytes, fibroblasts, myofibroblasts, neurons, adipocytes, chondrocytes and osteocytes. Compared with LSC, SSC undergo more population doublings. Stem cell biotechnologies represent new approaches for treating AAK through transplantation of bio-engineered limbal niche.

## Pr Neil Lagali (Sweden)

PhD

Neil Lagali is Associate Professor of Experimental Ophthalmology at Linköping University, Sweden, conducting research in corneal disease, regenerative medicine, tissue engineering, clinical imaging and angiogenesis. He is coordinator of the EU Horizon2020 project ARREST BLINDNESS, and a member of the Scientific Committee of Aniridia Europe. Dr Lagali has authored over 65 peer reviewed publications and serves as Associate Editor for *BMC Ophthalmology* and *Current Eye Research*.



### “Congenital Aniridia and the Ocular Surface”

Neil Lagali, Linköping University, Sweden

The cornea in aniridia is characterized by a progressive stem cell deficiency leading to breakdown of the limbal stem cell niche followed by a blinding conjunctivalization of the ocular surface called aniridia-associated keratopathy (AAK). Little, however, is known about the detailed mechanisms of this breakdown and the resulting impact on ocular surface characteristics. Through detailed imaging of the cornea in cohorts of persons with aniridia in Sweden, Norway, and Poland using in vivo confocal microscopy, ocular coherence tomography and slit lamp biomicroscopy, it was found that the limbal niche breakdown occurs in a specific pattern. Concurrently, ocular surface inflammation, neurodegeneration, neovascularization, and epithelial cell transformation occur in a staged, progressive manner. Interestingly, some of these changes may occur at the cellular level prior to clinical appearance of related signs or symptoms, and this has consequences for the medical and surgical management of the keratopathy. The latest imaging studies have recently been conducted in cohorts of children with aniridia of PAX6 and non-PAX6 origin in Germany and in Poland. These studies indicate early developmental changes in the anterior segment including specific expression of inflammatory, cellular and tissue-level changes in children and infants as early as 9 months of age. Detailed documentation of corneal pathology in young persons and protocols for ocular examination in children have been developed and will be presented. This knowledge enables us to investigate the time-dependent pathophysiology of AAK and relate this to genotypic (mutations) and phenotypic (eg. degree of iris hypoplasia) factors. This information may aid in the prognosis of the ocular surface condition in children as well as identification of a possible therapeutic window for application of novel therapies for maintaining a healthy and transparent ocular surface.

## **Pr Juan Alvarez de Toledo (Spain)**

MD, PhD, FEBO-CR

I am ophthalmologist working in Centro de Oftalmología Barraquer in Barcelona, Spain. I perform surgeries including corneal transplants, keratoprotheses, reconstructive procedures, cataract and clear lens extraction, and refractive surgical procedures. I have visited and treated many patients affected of congenital aniridia in Spain. I am member of the Scientific Committee of Aniridia Europe and coauthored some papers and book chapters about the disease.



### **“Histopathology Findings of Corneal Buttons in Congenital Aniridia Patients“**

#### **Description**

To evaluate the corneal button of primary penetrating keratoplasty of patients diagnosed with congenital aniridia.

#### **Materials / Patients and Methods**

A retrospective analysis of cases diagnosed with congenital aniridia was carried out. We analyzed 13 corneal buttons of 11 eyes with congenital aniridia. We only included those patients who underwent penetrating keratoplasty for the first time. The corneal buttons were analyzed for histological characteristics of the presence of vascularization, the presence or not of Bowman's layer, the thickness of the stroma and Descemet's membrane, and endothelium layer alterations.

#### **Results**

We found alterations in the epithelium and stroma in all patients, although this loss of architecture was not seen in Descemet's membrane and the endothelial population.

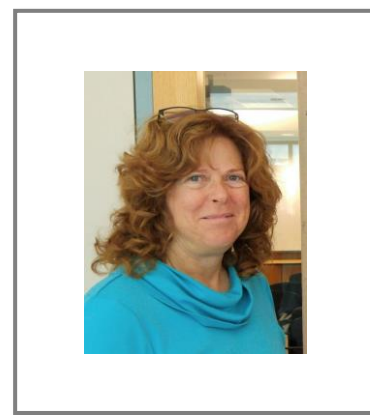
#### **Conclusion**

Patients with advanced congenital aniridic keratopathy may be good candidates for deep or superficial anterior lamellar keratoplasty for the preservation of normal endothelium and Descemet's membrane, along with limbal stem cell transplantation, to address epithelial and stromal pathology.

## Pr Elizabeth M. Simpson (Canada)

B.Sc., M.Sc., Ph.D., Professor in Medical Genetics at the University of British Columbia, Vancouver, Canada.

Dr. Simpson is a leading scientist in mammalian genetics and genomics. The goal of her research is to improve treatment for human disorders of the brain and eye. Currently, she is focused on the development of "MiniPromoters", human DNA control elements, and recombinant adeno-associated virus-based gene therapies.



### "Progress Towards Gene Therapy in the Mouse Aniridic Cornea"

Jack W. Hickmott<sup>1,2</sup>, Beatrice M. Tam<sup>3</sup>, Siu Ling Lam<sup>1</sup>, Orson L. Moritz<sup>3</sup>, Elizabeth M. Simpson<sup>1,2,3,\*</sup>

<sup>1</sup>Centre for Molecular Medicine and Therapeutics at BC Children's Hospital, University of British Columbia, Vancouver, British Columbia, Canada. <sup>2</sup>Department of Medical Genetics, University of British Columbia, Vancouver, British Columbia, Canada. <sup>3</sup>Department of Ophthalmology and Visual Science, University of British Columbia, Vancouver, British Columbia, Canada. \*Presenter

Aniridia is a rare blinding disorder caused by mutations in the gene paired box 6 (*PAX6*). Patients are born with poor vision, and vision loss advances in early adulthood primarily due to glaucoma (typically due to elevated intraocular pressure) and aniridia associated keratopathy (opacification of the cornea and thinning of the epithelium). Of these, keratopathy is the least well managed. Here we conducted tests of *PAX6* gene delivery, aimed towards the development of a gene therapy for the aniridic cornea. To facilitate detection of the therapeutic *PAX6* protein, we fused a 3xFLAG-tag (22 amino acid artificial antigen) to either the amino (N) or carboxyl terminus of wild-type (Wt) *PAX6* protein. We tested the functionality of the resulting proteins by their ability to generate ectopic eyes in *Xenopus laevis*. We chose to move forward with the N-terminal tagged 3xFLAG/*PAX6* protein, which did not significantly differ in the number of ectopic eyes produced compared to Wt *PAX6*. We then cloned either emerald green fluorescent protein (EmGFP) or 3xFLAG/*PAX6*, driven by the ubiquitous small chicken beta-actin promoter/CMV enhancer (smCBA) and stabilized by the woodchuck hepatitis virus post-transcriptional regulatory element (WPRE), into a single-stranded recombinant adeno-associated virus (rAAV) genome. Virus was packaged into rAAV capsid 9 for testing in the Small eye (*Sey*) mouse model of aniridia, on the B6129F1 defined hybrid background. Intrastromal injection (injection into the stroma of the cornea) is a clinically used corneal delivery method for antibiotics and antifungals. We found that intrastromal injection of rAAV9 smCBA-EmGFP-WPRE resulted in broad transduction of the cornea, including the stroma and epithelium, at one week. By two weeks, EmGFP stromal expression persisted but, as expected for this high turn-over tissue, epithelial expression waned. Importantly, EmGFP had no impact on corneal thickness. Therapeutically, we found that one week after intrastromal injection of smCBA-3xFLAG/*PAX6*-WPRE, *Sey* eyes had significantly thicker corneal epitheliums than untreated, and EmGFP treated control *Sey* eyes ( $p < 0.005$ ). Furthermore, the treated aniridic corneal epithelia were not significantly thinner than those of wild type mice. However, this effect was found to subside two weeks after injection. The success of transient *PAX6* expression in *Sey* mice paves the way for future work including construct, dose, and delivery optimization, towards development of a corneal gene therapy for aniridia.

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