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Short REPORT

ERN-EYE

4th SCIENTIFIC WORKSHOP: MODELS FOR RARE EYE DISEASES

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This document includes :
- *The short report, published on the ERN-EYE website*
- *The results of the satisfaction survey as annex 1*
- *The program of the event as annex 2*

European Reference Network
for rare or low prevalence complex diseases
Network Eye Diseases (ERN-EYE)

25th to 26th November 2021
Dublin, Ireland

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ERN-EYE WORKSHOP ON EYE MODELLING

ERN-EYE Project Management Team

Introduction

Scientific Workshop on Eye Modelling– November 25th- 26th, 2021

Following three previous scientific ERN-EYE workshops devoted to ontology, to genetic testing and to novel therapies, ERN-EYE has organized a fourth scientific workshop on "Eye Modelling" from 25th to 26th November in Dublin, Ireland.

After nearly 2 years of 100% remote events, this meeting took place in a hybrid format and gathered nearly 90 people, remotely or one site. These included the full members of ERN-EYE, the affiliated members and the future members of the network, ePAG members as well as prestigious invited international speakers.

The workshop was opened by the organizing committee composed by David Keegan, host of this ERN-EYE workshop and chair of the Transversal Working Group 5 on Low Vision, Daily Life and Patients Groups, Elfride de Baere, chair of the Transversal Working Group 6 on Genetic Diagnostics and Hélène Dollfus, ERN-EYE coordinator.

The first day of the workshop was devoted to the field cornea and anterior segment modelling and was followed by modelling of retinal inherited diseases that continued on the second day. Over the two days the most recognized European and international experts presented and discussed the latest modelling assays from the zebrafish and xenopus to mice and pigs. A focus was made on iPSC and organoids and their implications for the rare eye disease patients. Optogenetics promising avenues were also presented including a first in human investigation. All these models were shown to be useful for diagnosis as well as therapy developments in many ways.

Thanks to all these prestigious speakers, this workshop was the opportunity to discuss the progress and challenges in Eye Modelling and latest advances and technologies in the field.

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ERNs in brief

European Reference Networks (ERNs) are unique and innovative cross-border cooperation platforms between specialists for the diagnosis and treatment of rare or low prevalence complex diseases.

ERNs are virtual networks bringing together healthcare providers across Europe to tackle complex or rare medical conditions that require highly specialized treatment and a concentration of knowledge and resources. They are being set up under the EU Directive on Patients' Rights in Healthcare (2011/24/EU), which also makes it easier for patients to access information on healthcare and thus increase their treatment options.

The ERNs are supported by European cross-border telemedicine tools, and can benefit from a range of EU funding mechanisms such as the "Health Program", the "Connecting Europe Facility" and the EU research program "Horizon 2020".

DAY 1 - OPENING SESSION BY ORGANIZING COMMITTEE

General objectives of the meeting

Hélène Dollfus, ERN-EYE coordinator, David Keegan, host of this ERN-EYE workshop and chair of the Transversal Working Group 5 on Low Vision, Daily Life and Patients Groups, Elfride de Baere, chair of the Transversal Working Group 6 on Genetic Diagnostics

The workshop was opened by the organizing committee composed by David Keegan, host of this ERN-EYE workshop, Elfride de Baere, and Hélène Dollfus, ERN-EYE coordinator. Thay welcomed all attendees, online or on-site for the first ERN-EYE hybrid meeting.

They introduced the topic of the meeting and highlighted the importance of these models for eye research , from the physiopathology to the development of new therapies.

SESSION 1 MODELLING THE CORNEA AND ANTERIOR SEGMENT

Modelling the cornea for Fuchs and Other Corneal Dystrophies (Online)

Albert Jun, The Johns Hopkins Hospital, Baltimore, USA

Albert Jun, from Baltimore (USA), described mouse models of corneal dystrophies and how Nobel Prizes and ongoing research could lead to better approaches for corneal dystrophies. He explained that corneal dystrophies can be modelled in the mouse with variable fidelity and that mouse models of corneal dystrophies are valuable tools for elucidation of pathogenesis and therapeutic development. CRISPR has been used to treat a mouse model of Fuchs dystrophy and similar approaches could be used for other corneal dystrophies.

Modelling anterior segment disorders in zebrafish (Online)

Elena Semina, Medical College of Wisconsin, Wisconsin, USA

Research interests of Elena Semina are the genes involved in embryonic development with particular focus on ocular and cranofacial development. She presented us her results on MAB21L1 gene implied in Aniridia and PITX2 and FOXC1 genes in Axenfeld-Rieger syndrome in the Zebrafish.

Zebrafish is an important model for developmental ocular disorders including anterior segment dysgenesis phenotypes. She demonstrated that zebrafish mutants for PITX2, FOXC1 and MAB21L1 are excellent models for related human disorders.

Utilising patient-derived cell systems to model mechanisms of corneal endothelial dystrophies

Alice Davidson, UCL Institute of Ophthalmology, London, UK

Alice Davidson, from London (UK), spoke about utilising patient-derived cell systems to model mechanisms of corneal endothelial dystrophies.

She explained that WGS and digging into non-coding regions is necessary in a diagnostic work-up of patients with rare eye diseases.

SESSION 2 MODELLING RETINAL DISEASES I

Modelling rare eye diseases in Xenopus tropicalis using CRISPR-based genome editing methods

Kris Vleminckx, UGent, Gent, Belgium

Kris Vleminckx, from Gent, Belgium, talked about modelling rare eye diseases in Xenopus tropicalis using CRISPR-based genome editing methods. He explained that the recent advent of CRISPR/Cas9 as a straightforward genome-editing tool has allowed the establishment of the first bona fide genetic cancer models within the diploid aquatic model organism Xenopus tropicalis. He demonstrated the methods for targeting tumor suppressors with the CRISPR/Cas9 system in the developing X. tropicalis embryo. He illustrated genotyping and phenotyping of the resulting tumor-bearing F0 mosaic mutant animals (crispants).

The diversity of disease models for ocular disorders (Online)

Jane Farrar, Trinity College Dublin, Ireland

Jane Farrar, from Dublin (Ireland), explained that retinitis pigmentosa (RP) is the group of hereditary conditions involving death of retinal photoreceptors and represents the most prevalent cause of visual handicap among working populations in developed countries. She provided an overview of the molecular pathologies associated with such disorders, from which it becomes clearly apparent that RP is one of the most genetically heterogeneous of hereditary conditions for which molecular pathologies have been elucidated so far. While heterogeneity of such magnitude would appear to represent a major impediment to the development of therapeutics, mutation-independent approaches to therapy are being developed to effectively by-pass such diversity in genetic aetiology. The implications of such technologies in terms of therapeutic intervention in RP, and indeed other genetically heterogeneous conditions, will be addressed.

Restoring vision (Online)

Botond Roska, Institute of Molecular and Clinical Ophthalmology, Basel, Switzerland

Botond Roska explained that restoring vision to the blind by retinal repair has been a dream of medicine for centuries, and the first successful procedures have recently been performed. Although we are still far from the restoration of high-resolution vision, step-by-step developments are overcoming crucial bottlenecks in therapy development and have enabled the restoration of some visual function in patients with specific blindness-causing diseases. Botond Roska discussed the current state of vision restoration and the problems related to retinal repair. He described new model systems and translational technologies, as well as the clinical conditions in which new methods may help to combat blindness.

DAY 2 – SESSSION 3 MODELLING RETINAL DISEASES II

"Fishes to Dishes": Preclinical models for Inherited Retinal Degeneration and Uveal Melanoma

Brendan Kennedy, University College of Dublin, Dublin, Ireland

Brendan Kennedy, from Dublin (Ireland), explained that Zebrafish is a powerful model to discover ocular drugs. Worldwide, 1 in 2000 people suffer from inherited retinal dystrophies (IRD). Individuals with IRD typically present with progressive vision loss that ultimately results in blindness. Unfortunately, effective treatment options are not widely available due to the genetic and clinical heterogeneity of these diseases. There are multiple gene, cell, and drug-based therapies in various phases of clinical trials for IRD. In his study, Brendan Kennedy evaluated the potential of selective histone deacetylase 6 inhibitors to preserve retinal morphology or restore vision in zebrafish.

In vivo models of Stargardt macular degeneration as a tool for testing gene therapy approaches

Ivana Trapani, Telethon Institute of Genetics and Medicine, Pazzuoli, Italy

Ivana Trapani from Pazzuoli in Italy, talked about *in vivo* models in Stargardt disease. She explained that effective editing of the ABCA4 gene can be obtained in both ABCA4 KO and Cas9-injected pigs. This leads to reduction of ABCA4 protein expression, although at variable levels in Cas9-injected pigs. There is no significant retinal degeneration in both ABCA4 KO and Cas9-injected pigs and significant increase in lipofuscin accumulation in both ABCA4 KO and Cas9-injected pigs. Finally, AAV-intein treatment successfully restored ABCA4 expression in ABCA4 KO pigs Compare AAV-mediated vs WT ABCA4 levels in pigs.

Animal work in preparation for first in man trials for novel treatments

Lyndon Da Cruz, Moorfields Eye Hospital, London, UK

Lyndon Da Cruz presented the animal work in preparation for first in man trials for novel treatments. The field has developed over the past 30 years with advances coming from a large body of animal work and more recently a considerable number of human trials. Enormous progress has been made with the potential for at least partial restoration of visual function in both animal and human clinical work. Diseases that have been treated with RPE transplantation demonstrating partial reversal of vision loss include primary RPE dystrophies such as photoreceptor dystrophies as well as complex retinal diseases such as atrophic and neovascular age-related macular degeneration.

Large animal models for IRDs (Online)

Simon Petersen-Jones, Michigan State University, Michigan, USA

Simon Petersen-Jones explained that studies utilizing large animal models of inherited retinal degeneration (IRD) have proven important in not only the development of translational therapeutic approaches, but also in improving our understanding of disease mechanisms. The dog is the predominant species utilized because spontaneous IRD is common in the canine pet population. Cats are also a source of spontaneous IRDs. Other large animal models with spontaneous IRDs include sheep, horses and non-human primates (NHP). The pig has also proven valuable due to the ease in which transgenic animals can be generated and work is ongoing to produce engineered models of other large animal species including NHP. These large animal models offer important advantages over the widely used laboratory rodent models. The globe size and dimensions more closely parallel those of humans and, most importantly, they have a retinal region of high cone density and denser photoreceptor packing for high acuity vision. Laboratory rodents lack such a retinal region and, as macular disease is a critical cause for vision loss in humans, having a comparable retinal region in model species is particularly important.

Stargardt disease patient-derived photoreceptor precursor cells reveal retina-specific splice defects in ABCA4 RNA (Online)

Frans Cremers, Radboud University, Nijmegen, The Netherlands

Frans Cremers, from Nijmegen (Netherlands), argued that the majority of pseudoexon insertions can be shown using midigenes in HEK293T (kidney) cells. Photoreceptor progenitor cells and retinal organoids partially mimic retina-specific splicing. Retina-specific splicing can be due to new or alterated exonic splicing enhancer or silencer motifs. Finally, in silico prediction tools for retina-specific splicing need to be improved.

Unrecognized types of pathogenic variants underlying inherited retinal diseases (Online)

Susanne Roosing, Radboud University Medical Centre, Nijmegen, The Netherlands

Susanne Roosing, from Nijmegen (Netherlands), explained that RP17-SVs are an important cause of adRP and will most likely explain a significant portion of unsolved adRP families. Pathogenic RP17-SVs result in an altered TAD structure leading to increased retinal expression of GDPD1 and adRP (RP17). This study highlights the importance of SVs as a genomic mechanism in unsolved Mendelian disease.

Structural Variants Create New Topological Associated Domains and Ectopic Retinal Enhancer-Gene Contact (Online)

Suzanne de Bruijn, Radboud University Medical Centre, Nijmegen, The Netherlands

Suzanne de Bruijn, from Nijmegen, Netherlands, explained that the cause of autosomal-dominant retinitis pigmentosa, which leads to loss of vision and blindness, was investigated in families lacking a molecular diagnosis. The study, launched in collaboration with several institutes highlights the importance of SVs as a genomic mechanism in unsolved Mendelian diseases.

Organs-on-Chips for Retinal Disease Modelling

Andries van der Meer, Faculty of Science and Technology of the University of Twente, The Netherlands

Andries van der Meer, from Twente (Netherlands) talked about Organs-on-Chips for Retinal Disease Modelling. For him, an organ-on-a-chip is like an avatar of a patient and it is a controlled reverse engineering of human pathophysiology. He presented his recent results with this perspective.

Combining genome editing and retinal organoids to understand and treat Usher syndrome

Vasiliki Kalatzis, The Institute for Neurosciences of Montpellier, Montpellier, France

Vasiliki Kalatzis, from Montpellier (France), presented how retinal organoids are generated and characterized, and how they can help to understand retinal diseases such as USH2A-disease. She proposes to combine genome editing and retinal organoids to understand and treat Usher syndrome. There are different phenotypes in USH2A-RP and USH2A-USH organoids and the differential disease mechanism may explain hearing loss USH2A-USH. She explained that CRISPR/Cas9 isogenic correction is essential for validating phenotype and it needs Valuable prognostic tools for studying pathogenicity novel USH2A variants.

Faithful reproduction of a retinitis pigmentosa phenotype from human iPS cell- derived retinal organoids (Online)

Olivier Goureau, Institut de la Vision, Paris, France

Olivier Goureau, from Montpellier (France), explained that Animal models (when they exist) are not always very informative about the "pathogenicity" of the disease in humans. There are tools to understand and decipher molecular and cellular mechanisms underlying RP disease and tools to validate innovative gene therapies approaches: CRISPR/Cas9 (correction or edition) or AAV-based gene augmentation.

Using patient specific retinal organoids to understand the development of Retinoblastoma and design of therapeutic strategies

Majlinda Lako, Newcastle University, Newcastle, UK

Majilinda Lako, from Newcastle (UK), reminded that Retinoblasma is a childhood cancer of the developing retina, the light-detecting tissue of the eye. There is a lack of a mouse disease model: Rb1+/- mice do not develop retinoblastoma, Rb1-/- is embryonic lethal. NGS studies have identified a high frequency (46%) of additional somatic and likely pathogenic alterations beyond RB1 biallelic inactivation. Inter and intra tumour heterogeneity reported in a few cases. Finally, expression studies until today has been mostly done at bulk tissue level Single-Cell analyses present a unique opportunity to assess the intra and inter tumour heterogeneity.