10 PhDs on vision research to address the knowledge gaps to understanding, diagnosing and treating Inherited Retinal Diseases.

ProgRET is a Marie Skłodowska-Curie Doctoral Network (2024-2027), offering 10 PhD positions. The objective of ProgRET is to train a new generation of vision researchers specialising in inherited retinal diseases (IRD).

Eye diseases are among the most common inherited human disorders. Vision research has often blazed a trail for many disciplines to follow, giving a lead in multi-omics, stem cell biology, genome editing, animal models of disease, and the development of novel therapeutic approaches such as gene therapy. In recent years, human geneticists, have identified a large proportion of the genes implicated in IRD. Despite this progress the most important challenges in the IRD field relate to the diagnosis, understanding as well as therapy development of autosomal dominant IRD (adIRD). There are an estimated 925,000 affected individuals worldwide, representing 25%–40% of IRD. As we have demonstrated an emerging role for complex structural variants (SVs) and defects in non-coding regions such as non-coding RNAs and *cis*-regulatory elements in adIRD, we hypothesize that these may explain unsolved IRD cases and may represent novel targets for intervention.

The objective of ProgRET is to conduct advanced vision research that addresses the knowledge gaps to understanding, diagnosing and treating autosomal dominant inherited retinal disease (adIRD), using innovative approaches related to **genomics, transcriptomics, epigenomics, multi-omics, bioinformatics, gene regulation**, **retinal stem cell models, organoids, aquatic animal disease models**, and **therapy development.**

We will pursue the following specific research objectives: 1. To understand mechanisms of adIRD using retinal stem cell and aquatic animal models; 2. To advance diagnostics in adIRD using a single-molecule multi-omics framework; 3. To develop novel therapeutics for adIRD using RNA therapy and genome editing.

Application Deadline: 15 Mar 2024 - 23:59 (Europe/Paris)

Researcher Profile: First Stage Researcher (R1)

Research Field: ’(Human) Genetics’ ’Molecular Biology’ ’Biomedical research’ ’translational research’ ’integrative omics’ ’(Applied) Bioinformatics’ ‘Neurosciences’ ‘Gene therapy’

Type of Contract: Temporary

Job Status: Full-time

Offer Starting Date: Ranging from 1 May - 1 October 2024

Offer Description

ProgRET (European Training Program to Understand, Diagnose and Treat Autosomal Dominant Retinal Diseases) is a Marie Skłodowska-Curie Doctoral Network (2024-2027), offering **10 PhD positions.**

The PhD candidates will obtain PhD diplomas from reputed universities within the ProgRET project.

The network partners are:

- Center for Medical Genetics Ghent, Ghent University, Belgium
- Radboud University Medical Center Nijmegen, The Netherlands
- Institute for Ophthalmic Research, University of Tübingen, Germany
- Telethon Institute of Genetics and Medicine, Italy
- Institute for Neurosciences of Montpellier, University of Montpellier, France
- The Andalusian Centre of Developmental Biology, Spain
- Institute of Molecular Genetics, Czech Republic
- Evotec, Germany

Requirements

At the date of recruitment have a Masters' Degree (or equivalent) in one of the following research fields: '(Human) Genetics', 'Molecular Biology', 'Biomedical research', 'translational research', 'integrative omics', '(Applied) Bioinformatics', 'Neurosciences', 'Gene therapy'.

Languages: ENGLISH

Level: Excellent

Additional Information

Benefits

Marie Sklodowska-Curie PhDs are paid a competitive gross salary of 3,400 €/month, adjusted for their host country, a Mobility Allowance of 600 €/month and, for researchers who have a family, a Family Allowance of 660 €/month. All amounts are subject to deductions and taxes. Family is defined as persons linked to the researcher by (i) marriage, or (ii) a relationship with equivalent status to a marriage recognised by the national legislation of the country of the beneficiary or of nationality of the researcher, or (iii) dependent children who are actually being maintained by the researcher.

Eligibility criteria

To apply for one of these PhD positions, the applicant must fulfil the following conditions:

Have — **at the date of recruitment** — a Master's degree in Life Sciences, Biomedical Sciences, Biotechnology, Biochemistry, Biology, Bioinformatics, Systems Biology (or an equivalent diploma), Bioengineering.

Trans-national mobility: The applicant — **at the date of recruitment** — should not have resided in the country where the research training takes place for more than 12 months in the 3 years immediately prior to recruitment, and not have carried out their main activity (work, studies, etc.) in that country. For refugees under the Geneva Convention (1951 Refugee Convention and the 1967 Protocol), the refugee procedure (i.e. before refugee status is conferred) will not be counted as 'period of residence/activity in the country of the beneficiary'.

Be able to communicate **fluently in English** (at least B2-level speaking and writing).

How to apply:

To apply for one of these positions, **submit** to simone.dusseljee@radboudumc.nl **a single pdf document** containing:

- a detailed CV in EU format, including education, work experience, skills, dissertations, research interests, career objectives, and names and contact details of two referees, that can include the supervisor of the master thesis, willing to provide confidential letters of recommendation;
- a max. 1-page letter of motivation regarding the position(s) as well as the ProgRET network;
- a transcript of the master studies' grades (including the overall grade and an explanation of the grading system) and the master's thesis **if available**;

and indicate as TITLE your full name, add 3 KEYWORDS, and mention the PROJECT(S) you are interested in (**max 3**; DC1, ..., DC10, see below).

List of PhD topics

DC1: Impact of ageing on 3D genome architecture in adIRD model in killifish, an aquatic animal model
Supervisor: Prof. Juan R Martinez-Morales and Dr. Juan Tena
Host institute: The Andalusian Centre of Developmental Biology, Spain (www.cabd.es)
Secondments planned: Evotec, Germany; Ghent University, Belgium
Doctoral program: Biotechnology at the Pablo de Olavide University
Anticipated starting date: as soon as possible

Project description DC1@CSIC: Age is a critical factor for the onset of visual defects in most common IRD, including autosomal dominant IRD (adIRD). Although it is increasingly acknowledged that complex structural variants and non-coding defects play a role in the progression of IRD, the impact of aging on the regulatory landscape of IRD-associated genes has not been studied yet. Here, the PhD student will take advantage of unique features of the short-lived (<6 months) turquoise killifish (*Nothobranchius furzeri*) to investigate chromatin architecture and *cis*-regulatory maps in aging retinas. To this end, DC1 will use a combination of conformation capture methods: Hi-C (for global chromatin architecture) and HiChIP for H3K4me3 (that mark active promoters' interactions); in combination with chromium single-cell multi-omics (scRNA-seq + scATAC-seq) to interrogate the transcriptome and epigenome simultaneously in retinas from aged animals. In parallel, DC1 will develop animal models for adIRD in killifish, either by targeting disease genes [e.g. by establishing the transgenic line *Tg(rho:msRho-P23H-flag)*] or by mimicking structural or non-coding human variants in killifish [i.e. by CRISPR-Cas9 targeting of specific *cis*-regulatory elements or CTCF loops]. Chromatin architecture and *cis*-regulatory maps will be then examined in the disease models generated to identify regulatory modules potentially involved in aging and disease progression. The 3D topology in killifish will be compared with the 3D topology generated in human retina by DC5.

DC2: Characterization of stem cell models for defective spliceosome components in adIRD

Supervisor: Prof. David Staněk
Host institute: Institute of Molecular Genetics, Czech Republic
Secondments planned: Evotec, Germany; Institute for Neurosciences of Montpellier, France
Doctoral program: Developmental and Cell Biology at Charles University Prague
Anticipated starting date: October 1, 2024

Project description DC2@IMG: Mutations in several RNA splicing factors affect specific cells in the retina and lead to hereditary retinal degeneration - retinitis pigmentosa (RP). In addition, numerous mutations in retina-specific genes that also cause RP are found in introns and have potential negative effects on the splicing of these genes (e.g. RHO). Thus, splicing defects are key factors in the development of RP. Despite intensive research, the molecular mechanisms of cell-specific susceptibility to these mutations remain unclear. In this project, we plan to use relevant biomodels to study defects caused by RP mutations in splicing factors in target cell types. We will analyze the effect of RP mutations in different genes on in vitro generated human retinal organoids and retinal pigment epithelium. We will examine defects in RNA splicing and tissue-specific RNA production and identify genes with aberrant splicing that we will subsequently correct. We will also test the hypothesis that cellular sensitivity to RP mutations correlates with reduced expression of splicing factors. The results will allow us to identify potential treatments for RP.

DC3: High throughput screening assay for dominant BEST1 mutations to identify therapeutic compounds in an iPSC-RPE platform

Supervisor: Dr. N. Schwarz
Host institute: Evotec, Germany
Secondments planned: Institute of Molecular Genetics, Czech Republic; Institute for Neurosciences of Montpellier, France
Doctoral program: Tübingen University
Anticipated starting date: as soon as possible

Project description DC3@Evotec: Evotec is a biotech company with extensive expertise in cell therapy, drug screening and therapy development. In this program, the PhD student will differentiate patient or CRISPR-Cas9 derived iPSCs, carrying an autosomal dominant BEST1 mutation, into RPE cells using our unique in-house protocol. BEST1 mutant iPSC-RPE will be characterized for mislocalized BEST1, accumulation of lipofuscin and reduced calcium flux, and compared to wild type control iPSC-RPE. In addition, BEST1 mutant and control iPSC-RPE will be analyzed on transcriptome level with and without photoreceptor outer segment addition, to elucidate disease-relevant cellular pathways. Next, DC3 will establish a high-throughput compatible screening assay, which enables a proof of concept (POC) screening campaign with small, bio-annotated molecules to revert the observed cellular phenotypes. P8 has access to a large screening library of over 450,000 compounds and a subset of this library of approximately 1,000-2,000 compounds will be used. The development of high-throughput screening assay in an industry setting is essential to develop meaningful therapies for patients, since the quality requirements for such an assay are very high and require, at least partially, automation steps. Depending on the mechanisms, DC3 will evaluate other treatment modalities such as antisense oligonucleotides (AONs), in collaboration with DC7 and DC8 (WP3). We anticipate that correcting mutant BEST1 phenotypes, such as protein mislocalization, will restore cellular RPE function. Potential therapeutic compounds, identified through the POC screen, will be confirmed in the primary and secondary screening assays, as well as in complex 3D models, such as a co-culture of retinal organoid (ROs) and RPE cells. Any compounds identified in the POC screen will also be tested in this complex model and will therefore enhance the translatability of *in vitro* findings into the clinic.

DC4: Definition of transcriptional units of adIRD genes in human retina, RPE, PPCs and retinal organoids using long-read sequencing

Supervisor: Prof. S. Banfi
Host institute: Telethon Institute of Genetics and Medicine, Italy
Secondments planned: Phenopolis, UK (virtual); Ghent University, Belgium; Radboud University Medical Center Nijmegen, The Netherlands
Doctoral program: Biomolecular Sciences at University of Campania “Luigi Vanvitelli”
Anticipated starting date: October 1, 2024

Project description DC4@TIGEM: We have significantly contributed to the initial reconstruction of the architecture of the human retinal transcriptome and miRNome, in physiological conditions. By capitalizing on the above resources and competence, DC4 will characterize the transcriptional units of Inherited Retinal Disease (IRD) genes in the human retina using an integrated approach, including both meta-analysis of already available data and generation of new transcriptome datasets from retina

samples of both unaffected donors and retinal organoids generated from patient-derived iPSCs. Furthermore, DC4 will carry out co-expression analysis efforts using bioinformatic tools to reconstruct the gene networks that underlie the expression of IRD genes. Particular attention will be given to the identification and characterization of noncoding RNAs such as microRNAs and long noncoding RNAs that are significantly co-expressed with IRD genes. The activities of DC4 will aim at enhancing our understanding of the molecular basis of IRDs and at providing novel insights into the composition of the integrated gene networks that control retinal function.

DC5: Mapping of the 3D genome in human retina, RPE, and retinal organoids and non-coding variant interpretation in IRD
Supervisor: Prof. E. De Baere
Host institute: Ghent University, Belgium
Secondments planned: Phenopolis, UK (virtual); The Andalusian Centre of Developmental Biology, Spain; The Institute of Molecular and Clinical Ophthalmology Basel, Switzerland
Doctoral program: Medicine and Health Sciences at Ghent University
Anticipated starting date: as soon as possible

Project description DC5@UGent: Non-coding structural variants (SVs) and regulatory single-nucleotide variants (SNVs) are still underrepresented in the mutation spectrum of IRD, often due to an interpretation gap. A missing link is the 3D interaction between *cis*-regulatory elements (CREs) and their target genes within topologically associating domains or TADs. Using chromatin interaction mapping (Hi-C) we have recently shown a differential 3D genome architecture of human retina and retinal pigment epithelium (RPE). DC5 will investigate if C-technologies on clinically accessible tissues (LCLs/fibroblasts) and on retinal stem cells (photoreceptor precursor cells/PPCs, retinal organoids/ROs) can be used to evaluate the effect of SVs causing IRD on the 3D genome. Following up on our previous Hi-C studies on retina, RPE, LCLs and fibroblasts, DC5 will perform an adapted Hi-C protocol (low-C) on PPCs and ROs. Apart from short-read sequencing-based Hi-C, DC5 will apply long-read sequencing-based Pore-C to retina, RPE, PPCs and ROs to generate a reference dataset that allows to unravel regulatory mechanisms within IRD loci, and to resolve complex SV. DC5 will assess the conservation of the 3D architecture between these cell types on a genomewide scale with special attention to adIRD loci. Next, DC5 will use the generated 3D data and perform C-technologies on available patient-derived cells as a phenotyping tool to map and interpret non-coding SVs found in genome data of unsolved IRD patients. Finally, C-technologies and insights from non-coding SV interpretation will ultimately improve genetic diagnoses in IRD.

DC6: Single-molecule multi-omics framework for adIRD diagnosis
Supervisor: Dr. S. Roosing
Host institute: Radboud University Medical Center Nijmegen, The Netherlands
Secondments planned: Phenopolis, UK (virtual); The Institute of Molecular and Clinical Ophthalmology Basel, Switzerland; Ghent University, Belgium
Doctoral program: Radboud University Medical Center Nijmegen
Anticipated starting date: October 1, 2024

Project description DC6@RUMC: Although short-read whole genome sequencing (WGS) has increased the diagnostic yield in IRD, it is especially long-read WGS that can detect nearly all types of SVs, located in coding, non-coding, or repeat-rich regions. Integrating sequencing applications with technologies that provide insight into genomic organization without nucleotide resolution can be useful. Optical genome mapping (OGM) revealed ~7 times more SVs, compared to SR-WGS only and allows the detection of large SVs that are not captured by sequencing, as illustrated for IRD by P2-RUMC. DC6 will investigate the use of long read technologies to resolve the unexplained adIRD families. Known *in silico* prediction tools as well as new tools will be used to prioritize for variants of interest. Moreover, DC6 aims to assess known and novel putative splice affecting variants through midigene splice assays and complemented with long-read RNA sequencing methods for their pathogenicity to allow increasing of the classification from Variants of Unknown Significance to pathogenic. Next, the effect of SVs will be studied through Low-C in patient derived PPCs. Lessons learned from either pathogenic or benign SNVs and SVs and the utility of the long read approaches will be implemented into a framework that allows for improved genetic diagnosis in IRD.

DC7: Allele-specific invalidation of dominant-negative mutations in PRPH2
Supervisor: Prof. R. Collin
Host institute: Radboud University Medical Center Nijmegen, The Netherlands
Secondments planned: Ghent University, Belgium; Gulliver Biomed, Belgium
Doctoral program: Donders Institute for Brain, Cognition and Behaviour at Radboud University Nijmegen
Anticipated starting date: May 1, 2024

Project description DC7@RUMC: Central areolar choroidal dystrophy (CACD) is caused by autosomal dominant pathogenic variants in *PRPH2*, encoding a tetraspanin protein (peripherin-2) present in the photoreceptor outer segments. Whilst some *PRHP2* mutations act in via haploinsufficiency, other variants exert their pathogenicity via a dominant-negative mechanism. For the latter group, allele-specific degradation of the mutant transcript is expected to be of therapeutic benefit. Based on preliminary data for one recurrent missense mutation in *PRPH2*, as well as locus-sequencing data that identified common polymorphisms *in cis* with the mutation, P2-RUMC has identified a range of therapeutic targets. Here, PhD student DC7 aims to identify which of the many *PRPH2* mutations act in a dominant-negative manner, and in parallel will realize an allele-specific inhibition of the expression of the mutant protein via antisense oligonucleotides (ASOs). Via VIP (visible immunoprecipitation) assays DC7 will study the ability of mutant peripherin-2 to interact with other peripherin-2 and/or ROM1 proteins. In parallel, ASOs will be designed targeting the mutation and/or variants elsewhere in the gene that are *in cis* with the pathogenic variant. Allele-specificity and efficacy will first be measured in transfection assays, and later optimized in an established patient-derived RO model.

DC8: Allele-independent increase of protein translation for adIRD genes displaying haplo-insufficiency
Supervisor: Prof. F. Coppieters
Host institute: Ghent University, Belgium
Secondments planned: Evotec, Germany; Radboud University Medical Center Nijmegen, The Netherlands
Doctoral program: Medicine and Health Sciences at Ghent University
Anticipated starting date: as soon as possible

Project description DC8@UGent: IRD is at the forefront of gene therapy development. This PhD project aims to explore a novel therapeutic approach to increase protein expression of IRD disease genes that display haploinsufficiency by targeting *cis*-acting, non-coding elements. We previously performed both *in silico* and wet-lab analyses to identify *cis*-acting elements that modulate expression of IRD genes in the retina. DC8 will first functionally dissect novel *cis*-acting elements using *in vitro* reporter assays in cellular models. Next, DC8 will design and evaluate both antisense oligonucleotides and base editing tools to modulate *cis*-acting elements and as such increase protein expression in wild-type retinal models. Finally, the efficacy of the most promising therapeutic molecules will be assessed in patient-derived mutant cellular models and retinal organoids. This project will provide new scientific insights in IRD gene regulation by elucidating the function of novel *cis*-regulatory elements, and will evaluate a novel, mutation-independent therapeutic strategy to modulate IRD gene expression, which is highly valuable in view of the allelic heterogeneity that is typical for IRD.

DC9: Allele-specific, mutation-independent rescue of dominant negative acting IRD mutations by single gRNA-Cas variant genome editing
Supervisor: Dr. S. Kohl
Host institute: University of Tübingen, Germany
Secondments planned: Gulliver Biomed, Belgium; Institute for Neurosciences of Montpellier, France
Doctoral program: Mathematics-Natural Sciences at Eberhard Karls Universität Tübingen
Anticipated starting date: as soon as possible

Project description DC9@UT: Certain pathogenic variants in known inherited retinal disease related genes are known to cause autosomal dominant disease by gain-of-function pathomechanism. We anticipate to rescue the phenotype by specifically disrupting the aberrant allele via genome editing strategies. Specifically, we will focus on an allele-specific but mutation-independent design to overcome mutational heterogeneity. Within our research group we have identified frequent SNPs in these genes that are also commonly found heterozygously in the general population. PhD student DC9 will develop a cell-based reporter assay to unbiasedly screen for potent and specific gRNAs and Cas variants, and will effectively target such SNPs *in cis* with the respective mutation. DC9 will apply and further develop small and specific synthetic genome editing molecules that will be bioengineered to result in robust mutant allele-specific gene disruption, hereby leaving the wild-type allele of the gene intact. By shutting off the production of mutant protein, the project aims to rescue the disease phenotype in patient-derived cellular models and/or retinal organoids using clinically viable delivery tools (i.e. AAV particles).

DC10: Evaluating allele-specific invalidation therapies for autosomal dominant CRX mutations
Supervisor: Dr. V. Kalatzis
Host institute: Institute for Neurosciences of Montpellier, France
Secondments planned: Evotec, Germany; University of Tübingen, Germany
Doctoral program: Chemical and biological health sciences, University of Montpellier
Anticipated starting date: October 1, 2024

Project description DC10@INSERM: Carriers of dominant *CRX* pathogenic missense variants can present with different clinical phenotypes: Leber Congenital Amaurosis (LCA), cone-rod dystrophy (CRD) and retinitis pigmentosa (RP). Moreover, individuals with pathogenic variants can be asymptomatic (non-penetrance). Based on preliminary data, we hypothesise that this maybe due to aberrant splicing even in the case of missense variants not localized at canonical splice sites. To test this hypothesis, DC10 will develop a *CRX* minigene assay to investigate the effect on splicing of selected *CRX* missense variants. In parallel, DC10 will validate splicing effects in iPSC-derived retinal organoids from asymptomatic and symptomatic patients within the same family. Furthermore, DC10 will assay the effect of mutant allele-specific knockdown using CRISPR-Cas9-mediated genome editing and/or ASO-mediated exon skipping to further validate these results and as a potential therapeutic strategy. The work performed by DC10 could provide a prognostic tool for testing the pathogenicity of *CRX* variants and lead to the development of innovative therapeutics.

Additional comments

For more information on ProgRET and additional job details see http://progret.eu/

Where to submit your application: simone.dusseljee@radboudumc.nl

Deadline: March 15, 2024, after which we will review applications and interview candidates.

All questions about the vacancies and submission should be emailed to the project manager of ProgRet: simone.dusseljee@radboudumc.nl