Marie Curie Innovative Training Network

Training researchers to Diagnose, Understand and Treat Stargardt Disease, a Frequent Inherited Blinding Disorder

(StarT project)

Eye diseases are among the most common inherited human disorders. Around one third of the known genetic defects or syndromes involve the eye. Vision research has often blazed a trail for many disciplines to follow, giving a lead in omics, genome editing, stem cell biology, animal models of disease, and the development of novel therapeutic approaches such as gene therapy.

StarT will create an interdisciplinary and intersectorial European training network focusing on different aspects of autosomal recessive Stargardt disease (STGD1), a frequent inherited blinding disorder that affects an estimated 925,000 persons worldwide, representing more than one-third of all inherited retinal disorders. StarT research aims to uncover the regulation of its disease gene ABCA4 and its missing heritability, in order to develop novel treatments.

STGD1 is due to ABCA4 mutations, however up to 35% of STGD1 cases carries one or no ABCA4 coding mutation. New unconventional classes of ABCA4 mutations were recently discovered by us, the significance of which largely remains elusive. In order to understand the mechanisms triggered by these missing ABCA4 mutations and to design new therapies for STGD1 cases, challenging research questions will be addressed by the integration of unique skills from this network.

Early-Stage Researchers will perform cutting edge research using innovative and interdisciplinary approaches: (functional) genomics and transcriptomics, bio-informatics, CRISPR/Cas9 genome editing, generation of stem cell and animal disease models and design of new treatments.

Individual project descriptions

ESR1 Partner 4 (TIGEM): Identification of the gene networks that modulate ABCA4 expression
We have recently generated a comprehensive mRNA and microRNA (miRNAs) transcriptome of the human retina and have acquired expertise in co-expression analysis. ESR1 will capitalise on these resources to gain insight into the organisation of the ABCA4 transcriptional unit and into the dissection of the gene networks that participate in the modulation of the expression and function of ABCA4 in human retina. ESR1 will further define the genomic organisation of the ABCA4 gene using our vast RNA-seq dataset to identify and validate the presence of alternative transcript variants. By integrating already available and newly-generated transcriptomics data carried out both in human and in mouse, ESR1 will reconstruct the transcriptional gene networks that modulate ABCA4 expression in photoreceptors in both physiological and pathological conditions. ESR1 will pay particular attention to the contribution of miRNAs and will combine target predictions with our data on miRNA expression in the retina to select candidate miRNAs predicted to regulate ABCA4 and validate their biological relevance using in vitro and in vivo assays. **Supervisor**: Prof. Dr. S. Banfi.

ESR5 Partner 2 (RUMC): Identification and splice assays of deep-intronic ABCA4 variants in mono-allelic STGD1

Approximately 25% of STGD1 cases show one or no coding ABCA4 variant. Using ABCA4 locus sequencing, we and others identified deep-intronic variants. We focused on the identification of RNA splice defects and generated a complete set of Gateway-based splice vectors, denoted midigenes, that contain wild-type (WT) ABCA4 multi-exon segments of 4.7 to 11.7 kb. Using a mutagenesis protocol, we rapidly introduced new variants into these vectors and performed in vitro splice assays in HEK293T cells. We assessed the effect of all reported 47 non-canonical ABCA4 splice variants and tested 10 deep-intronic variants identified in 40 mono-allelic Dutch STGD1 cases. Splice defects were visualised by RT-PCR using primers annealing to flanking ABCA4 exons. For selected variants, we also confirmed their effect on patient-derived photoreceptor progenitor cells (PPCs). ESR5 will develop a cost-effective sequencing method for the ABCA4 locus using single molecule Molecular Inversion Probes (smMIPs), and sequence 400 mono-allelic STGD1 cases that have been recruited by P2-RUMC. Sequence data (variants) of ESR5, ESR6 and ESR7 will be compiled. Hundred variants predicted to affect splicing will be introduced into WT midigenes. The effect of selected variants will be analysed in patient-derived PPCs and retinal pigment epithelium (RPE) cells. **Supervisor:** Prof. Dr. F. Cremers. url: https://www.ru.nl/donders/research/theme-2-perception-action-control/research-groups-theme-2/blindness-genetics/

ESR10 Partner 10 (ProQR): Optimisation, delivery and tolerability of antisense oligonucleotides to treat STGD1 patients with the most common splice mutation in ABCA4

ProQR is currently in Phase IIa clinical development with a splice modulating AON (QR-110) targeting the deep-intronic c.2991+1655A>G mutation in LCA type 10. In addition, they have two additional AON programs in Usher syndrome targeted all exon-13 mutations (QR-421a) and c.7595-2144A>G (QR-411a), respectively, in pre-IND/CTA enabling studies. ProQR is also in the discovery phase with regards to an intronic ABCA4 mutation (c.5461-10T>C), one of the most frequent mutations underlying STGD1 that results in the exclusion of exon 39, or exon 39 and exon 40 together, from the mRNA, leading to a frameshift. ESR10 will continue this program and optimise the sequence and chemistry of the final molecule to generate a clinical development candidate. This lead candidate will be further optimised by determining the extent of target engagement using digital-droplet PCR, immune-profiling against human donor T-lymphocyte panels, delivery to photoreceptors tolerability and pharmacokinetic parameters following intravitreal injection of mouse and rabbit models. **Supervisor:** Prof. Dr. P. Adamson.

ESR11 Partner 4 (TIGEM): ABCA4 knockout pig as model for gene therapy in STGD1

Abca4-/- knockout mice are currently used as animal models of STGD1, but they recapitulate only some of the features of the disease, which might be due to the structure of the mouse retina, which largely differs from that of humans. The absence of an appropriate animal model severely limits both the understanding of STGD1 mechanisms as well as the testing of novel potential therapeutic strategies. Among non-primate mammals, the porcine eye shares many similarities with the human retina including anatomy, size, and a high cone/rod ratio. In addition, unlike in non-human primates or other large species, pig transgenesis is particularly advanced. ESR11 will generate a pig model of STGD1, by exploring either nuclear transfer from fibroblasts which have been genetically modified using TALEN technology in embryonic stem cells, or photoreceptor somatic gene transfer of CRISPR/Cas9 with adeno-associated viral vectors. The generation of in vivo models of STGD1 will provide unique tools for both studying the mechanism of STGD1 rod and cone cell death as well as testing new therapies including gene therapy approaches recently developed by us. **Supervisor:** Prof. Dr. A. Auricchio.

ESR13 Partner 7 (TCD): AAV vector delivery targeting common pathways of disease in STGD1

There is growing evidence that many different genetic forms of IRD share common disease mechanisms. Indeed, similar disease processes between ABCA4-associated STGD1 and age related macular degeneration (AMD) have been proposed. Loss of the ABCA4 transporter involves, among other disease mechanisms, a build-up of di-retinoid-pyridinium-ethanolamine (A2E), a vitamin A dimer that becomes trapped in the retinal pigment epithelium (RPE). A2E is a major component of lipofuscin, a hallmark of human STGD1, the Abca4-/- mouse model and AMD. In turn it has been clearly demonstrated that the ATP production capacity of mitochondria in RPE cells is greatly diminished in the presence of A2E. Here, a novel therapeutic strategy for STGD1 is proposed: methods to sustain the mitochondrial function and ATP production capacity of RPE cells in the Abca4-/- mouse model of STGD1 and in cell models of disease generated as part of the planned research program. ESR13 will generate AAV vectors expressing components to augment mitochondrial function and modulate oxygen consumption rates and ATP production. Methods to assess mitochondrial function will be employed and potential beneficial effects of delivery of AAV vectors targeting such common pathways of disease will be evaluated in cell and animal models of STGD1. **Supervisor:** Prof. Dr. J. Farrar.

Name applicant …….

Preference for one or more partner institutes and ESR research subjects:

No*	Fellow no.	Project Title	Partner Institute
	ESR 1	Identification of the gene networks that modulate ABCA4 expression	Telethon Institute of Genetics and Medicine, IT
	ESR 5	Identification and splice assays of deep-intronic ABCA4 variants in mono-allelic STGD1	Radboud University Medical Center Nijmegen, NL
	ESR 10	Optimisation, delivery and tolerability of antisense oligonucleotides to treat STGD1 patients with the most common splice mutation in *ABCA4*	ProQR Therapeutics, NL
	ESR 11	*ABCA4* knockout pig as model for gene therapy in STGD1	Telethon Institute of Genetics and Medicine, IT
	ESR 13	AAV vector delivery targeting common pathways of disease in STGD1	Trinity College Dublin, IE

* Please mark the preferred ESR projects with a number. (max 3!)

EUROPEAN CURRICULUM VITAE FORMAT

PERSONAL INFORMATION

Name	[SURNAME, other name(s)]
Address	[House number, street name, postcode, city, country]
Telephone	
Fax	
E-mail	
Nationality	
Gender	Male / Female
Date of birth	[Day, month, year]

WORK EXPERIENCE

• Dates (from – to)	[Add separate entries for each relevant post occupied, starting with the most recent.]
• Name and address of employer	
• Type of business or sector	
• Occupation or position held	
• Main activities and responsibilities	

EDUCATION AND TRAINING

• Dates (from – to)	[Add separate entries for each relevant course you have completed, starting with the most recent.]
• Name and type of organisation providing education and training	
• Principal subjects/occupational skills covered	
• Title of qualification awarded	
• Level in national classification (if appropriate)	

PERSONAL SKILLS AND COMPETENCES *Acquired in the course of life and career but not necessarily covered by formal certificates and diplomas*.	
MOTHER TONGUE	[Specify mother tongue]
OTHER LANGUAGES	
	[Specify language]
• Reading skills	[Indicate level: excellent, good, basic.]
• Writing skills	[Indicate level: excellent, good, basic.]
• Verbal skills	[Indicate level: excellent, good, basic.]
SOCIAL SKILLS AND COMPETENCES *Living and working with other people, in multicultural environments, in positions where communication is important and situations where teamwork is essential (for example culture and sports), etc.*	[Describe these competences and indicate where they were acquired.]
ORGANISATIONAL SKILLS AND COMPETENCES *Coordination and administration of people, projects and budgets; at work, in voluntary work (for example culture and sports) and at home, etc.*	[Describe these competences and indicate where they were acquired.]
AVAILABILITY	[Please indicate what would be the earliest date that you can start a new job]
ADDITIONAL INFORMATION	[Include here any other information that may be relevant, for example contact persons, references, etc.]
ANNEXES	[List any attached annexes.]