Master Biologie Moléculaire et Cellulaire 'BMC',
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Parcours : **Biologie et Développement Cellulaires 'BDC'**
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Unité INSERM ou CNRS ou Université : **UMR U1163** **Intitulé Equipe :** **Laboratoire de Génétique ophtalmologique** **ED d'appartenance :** **BioSPC** **Responsable de l'Equipe :** **Jean-Michel Rozet**	**Responsable du Stage :** **Isabelle Perrault** **Contacts** Adresse : Institut Imagine 24bd du Montparnasse 75015 PARIS Email : isabelle.perrault@inserm.fr Tel :0142754307

Functional characterization of new gene responsible for early onset and severe retinal dystrophy (EOSRD)

Early-Onset Severe Retinal Disease (EOSRD) are the leading cause of incurable blindness in children. These diseases, which are variable on the clinical, genetic and pathophysiological terms. The identification of responsible genes and genotype-phenotype correlations has significantly improved the management of these diseases. However, after performing next-generation sequencing on a gene panel associated with retinal dystrophies, we were able to diagnose 87% of the EOSRD cohort. Unfortunately, 13% of the families could not benefit from these advances due to a negative molecular diagnosis. Mutations in the non-coding regions of the identified genes are to be envisaged and work is being carried out to search for them. But new genes are also to be discovered, as demonstrated by the recent identification in our laboratory of *TUBB4B, RIMS2* and *GPATCH11* the mutations of which cause a syndromic forms of early-onset severe retinal disease.

Recently, through whole-exome and whole-genome sequencing in families with EOSRD, we identified **new candidate genes**. We initially focused on a consanguineous family with a single affected individual who carried a homozygous nonsense variant. This gene encodes a protein that is highly expressed in the brain and contains two C2 domains, which are commonly found in proteins involved in signal transduction or membrane trafficking. Its expression pattern and similarity to other proteins suggest it may play a role in synaptic functions. The identification of a nonsense variant, along with the expression and potential function of this protein, positions this gene as a promising candidate for retinal dystrophy. The patient's fibroblasts were requested, allowing us to validate the impact of the variant on RNA and protein. Additionally, we studied a second consanguineous and multiplex family, where linkage analysis revealed a homozygous region on chromosome 5, and genome sequencing identified non-coding or missense variant in only two genes within this region, respectively. The first encodes a importin, a member of transport receptors that mediate nucleocytoplasmic transport of protein and RNA cargoes. The second encodes a protein which plays a critical role in cellular cholesterol homeostasis. The nature of the identified variants and the presumed function of the proteins do not strongly position these two genes as candidates. This is why we initiated an RNA-Seq analysis on the patient's fibroblasts to combine genome analysis with **transcriptome analysis**, in order to identify dysregulated genes located within the homozygosity region.

The goal of this internship is **to validate the role of the identified variants in the pathology and to investigate the function of the new candidate gene in retinal physiology**. As demonstrated in our recent publications, we have a variety of tools to accomplish this. Firstly, we have patient-derived fibroblasts, which enable us to assess RNA and protein localization and expression using techniques such as qPCR, immunofluorescence, microscopy, expansion microscopy, transcriptomics, Western blot, and proteomics. The creation of an animal model (mouse or zebrafish) could also be considered if the internship leads to a PhD.

Publications de l'équipe relatives au projet de stage

- Dodd DO, Mechaussier S … Rozet JM, Perrault I, Mill P. Ciliopathy patient variants reveal organelle-specific functions for TUBB4B in axonemal microtubules. Science. 2024 Apr 26;384(6694):eadf5489.
- Zanetti A, … Rozet JM, Perrault I. GPATCH11 variants cause mis-splicing and early-onset retinal dystrophy with neurological impairment. Nat Commun. 2024 Nov 21;15(1):10096
- Mechaussier S, …Perrault I. Loss of function of RIMS2, a synaptic membrane exocytosis gene, causes a syndromic congenital cone-rod synaptic disease with neurodevelopmental and pancreatic involvement. Am J Hum Genet. 2020 May 24
- Luscan R, Mechaussier S, … Perrault I. Mutations in TUBB4B Cause a Distinctive Sensorineural Disease. Am J Hum Genet. 2017 Dec 7;101(6):1006-1012.