PASTEUR PARIS UNIVERSITE (PPU) INTERNATIONAL DOCTORAL PROGRAM 2020

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PROJECT

- **FILE #12**
- **ACRONYM:** REnAL Intronic SplicE (REALISE)
- **TITLE:** Identification and characterization of intronic variants in hereditary renal diseases

LABORATORY

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LABORATORY PRESENTATION AND RESEARCH TOPICS

- **SUPERVISOR HDR:** MOLLET Géraldine (PhD, HDR), geraldine.mollet@inserm.fr

- **SPECIFY THE TEAM NAME:** Laboratory of hereditary kidney diseases

- **CO-SUPERVISOR:** DORVAL Guillaume (MD-PhD)

WEBSITE OF THE TEAM: https://www.institutimagine.org/fr/193

DESCRIPTION OF THE PROPOSED PROJECT

- **KEYWORDS:** rare renal diseases / intronic variants / induced pluripotent stem cells (iPSCs) / kidney organoids

- **ABSTRACT**

Whole exome sequencing (WES) do not allow identification of deep intronic variants. In case of hereditary podocytopathies, a second molecular event is often missed in autosomal recessive forms. The aim of this thesis is to set up an integrative approach combining whole genome sequencing and RNA-Seq from tissues relevant to the disease or from organoids derived from modified iPSCs to identify and characterize missing variants not identified by WES.

- **DESCRIPTION OF THE PROJECT**

Our group is interested in the identification and characterization of novel genes and/or novel mutations in genes already known to be responsible for hereditary podocytopathies, a group of rare renal disorders, using the latest cutting-edge technologies, such as whole exome sequencing (WES), CRISPR/Cas9 technology, induced pluripotent stem cells (iPSCs) and kidney organoids.

The advent of NGS, and mainly WES in the last decade has allowed the discovery of many exonic variants or variants involving splice sites responsible for monogenic diseases. However, it does not allow the identification of potential deep intronic variants missed by exome or panel sequencing, which could lead to abnormal splicing events, thus preventing molecular

diagnosis in a significant number of patients (**1**). These variants can be identified by whole genome sequencing (WGS), but interpretation of their pathogenicity is often delicate and requires additional functional studies. The aim of this thesis would be to set up an integrative approach combining WGS and RNA-Seq from tissues relevant to the disease or from organoids derived from modified iPSC line to identify and characterize intronic variants using the model of hereditary podocytopathies (HP), especially in autosomal recessive (AR) forms. HP are characterized by chronic proteinuria or even a steroid-resistant nephrotic syndrome (SRNS) and are due to genetic abnormalities intrinsic to the podocyte, a highly differentiated renal glomerular cell that is crucial for the maintenance of a functional glomerular filtration barrier. Usual genetic exploration techniques have revealed a monogenic origin in 30% of patients with SRNS with an important age gradient from 70% in congenital forms to 11% after the age of 13 years (**2**). It is obvious that in some cases, the usual genetic exploration techniques do not allow to establish a molecular diagnosis despite the strong clinical suspicion. Therefore, our laboratory recently developed an approach to identify such intronic variants in a pilot project involving 11 patients with X-linked Alport Syndrome, a chronic renal glomerulopathy, and no pathogenic variant identified in the responsible gene (*COL4A5)* by usual techniques. RNA-sequencing (RNA-Seq) coupled with genomic DNA sequencing of *COL4A5* allowed us to successfully identify a molecular cause in 9 of them (82%). Furthermore, in a patient with AR SRNS and harboring a single exonic mutation in the *NPHS2* gene, we recently identified by WGS a second intronic variant that needs to be characterized.

The aim of the PhD project is thus to investigate and characterize deep intronic variants in a well-defined population with a confirmed diagnosis of AR-SRNS with a single mutation identified in known SRNS genes. The project will comprise the following steps carried out in parallel:

1- Characterization of the *NPHS2* intronic variant already found by WGS using RNA-seq. This will be achieved by introducing this variant by CRISPR/Cas9 in a control iPSC line (see below).

2- Analysis of genome sequencing in 10 trios at the Imagine Institute on the Genomic Platform to identify the rare intronic variants. RNA-Seq will be performed on relevant material, either from patient fibroblasts/lymphocytes in the case of SRNS gene expressed in skin/blood, or in patient kidney, if available, or in podocytes isolated from kidney organoids. For the latter, intronic variants will be individually introduced in a control iPSC line using the CRISPR/Cas9 technology, an efficient technique already performed routinely in our laboratory. Then, these gene-edited iPSCs will be differentiated into 3D kidney organoids using a well-established protocol that allows the differentiation of iPSCs into multipotent renal cell progenitors that can subsequently form nephron-like structures containing podocyte-like cells (**3**). Podocytes will be isolated from these kidney organoids using fluorescence-activated cell sorting (FACS) with podocyte-specific markers (podocalyxin/nephrin).

Bioinformatics analyses will be performed in partnership with the bioinformatics platform of the Imagine Institute (P. Nitschke and N. Cagnard), using the algorithms being already developed for the pilot project on Alport Syndrome. These algorithms that score all potential non-canonical junctions (exon/exon or exon/intron) allowing the identification of abnormal splicing events would need to be improved for the specific studied genes and in the long-term to non-targeted genes. The coupled analysis of RNA-Seq and genome sequencing data will allow a sufficient stringency for the identification and the choice of candidate variants. In addition, this approach could be used to perform transcriptome profiling aiming to identify specific molecular signature for HP, and to a larger extent to other rare hereditary diseases.

- **REFERENCES**

(**1**) Horinouchi T. et al., **2018**. Detection of Splicing Abnormalities and Genotype-Phenotype Correlation in X-linked Alport Syndrome. *J Am Soc Nephrol*. 29(8):2244-2254. doi: 10.1681/ASN.2018030228.
(**2**) Sadowski CE. et al., **2015**. A single-gene cause in 29.5% of cases of steroid-resistant nephrotic syndrome. J Am Soc Nephrol. 26(6):1279-89. doi: 10.1681/ASN.2014050489.
(**3**) Morizane R. et al, **2015**. Nephron organoids derived from human pluripotent stem cells model kidney development and injury. *Nat Biotechnol.* 33(11):1193-200. doi: 10.1038/nbt.3392.

EXPECTED PROFILE OF THE CANDIDATE

EXPERIENCE REQUIRED

The applicant should have a high interest in large-scale data analysis (RNA-seq / WGS). Good knowledge of genetics, cell and molecular biology.