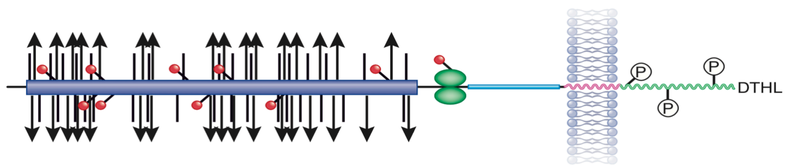
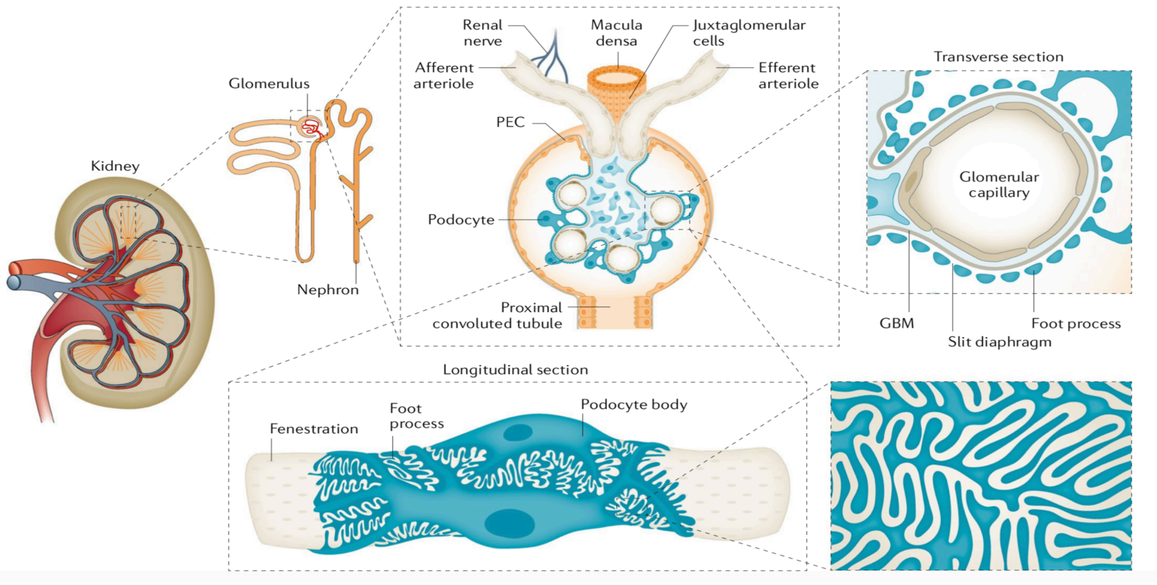
Cellular mechanisms of disease in nonsense mutations of the gene PODXL associated with autosomal dominant podocytopathies.

Marie Dirix
Tutors: Guillaume Dorval et Corinne Antignac
Laboratory of Hereditary Kidney Diseases, Institut Imagine





Introduction

- Podocalyxine (gene: PODXL) plays a key role in the morphogenesis and the maintenance of cell architecture of podocytes.[1]
- Recent pedigree studies linked heterozygous nonsense mutations of PODXL with adult-onset autosomal-dominant focal segmental glomerulosclerosis (FSGS).[2]
- This phenomenon has been explained by haplo-insufficiency through nonsense-mediated RNA decay.

BUT:

- Missense mutations have been described that do not alter the quantity of the protein but are nevertheless associated with proteinuria
- Numerous nonsense variants of PODXL exist in exome databases of healthy controls.

Hypothesis: dominant negative effect

We suspect that certain variants will lead to protein truncation and block protein trafficking from the endoplasmatic reticulum (ER)

Methodology

Objective 1: Demonstrate that certain nonsense mutations that lead to RNA decay do not cause the phenotype

- Nonsense mutations identified in 3 patients and 1 healthy person
- IPS → CRISPR-Cas9 → renal organoids → RNA seq
- RNA sequencing will identify the variants that lead to RNA decay.

Objective 2: Search for a dominant negative effect in the variants that do not lead to RNA decay but are associated with the phenotype.

- Transfection of immortalised podocytes with plasmids containing the chosen variants
- Confocal microscopy: localisation of the wild-type (WT) versus the mutant protein?
- Quantification of specific markers of ER stress through western blot.

1. Takeda T et al. Expression of podocalyxin inhibits cell-cell adhesion and modifies junctional properties in Madin-Darby canine kidney cells. *Mol Biol Cell. 2000;11(9):3219-3232.*
2. Barua M et al. Exome sequencing and in vitro studies identified podocalyxin as a candidate gene for focal and segmental glomerulosclerosis. *Kidney Int. 2014 Jan;85(1):124-33.*
3. Refaeli I et al. Distinct Functional Requirements for Podocalyxin in Immature and Mature Podocytes Reveal Mechanisms of Human Kidney Disease. *Sci Rep. 2020 Jun 10;10(1):9419.*

Contact: marie.dirix@hotmail.com