ALLELE-SPECIFIC METHYLATION BY NANOPORE SEQUENCING IN COMPLEMENT-DEPENDENT THROMBOTIC MICROANGIOPATHIES

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Introduction and background

The emergence of **nanopore-type ultra-fast genomic sequencing** appears to offer the potential for rapid molecular diagnosis of patients with **thrombotic microangiopathies** (TMA).

TMA is a disease characterized by **incomplete penetrance**, which very often explains its sporadic mode of expression. For the time being, several hypotheses can be put forward, including an **epigenetic mechanism**, such as a **methylation anomaly** in the allele carrying the variation, which has not yet been reported, and which interests us here.

The team has just validated the methylation detection protocol in-house on a human podocyte cell line, where the methylation difference is easily visualized (Figure 1).

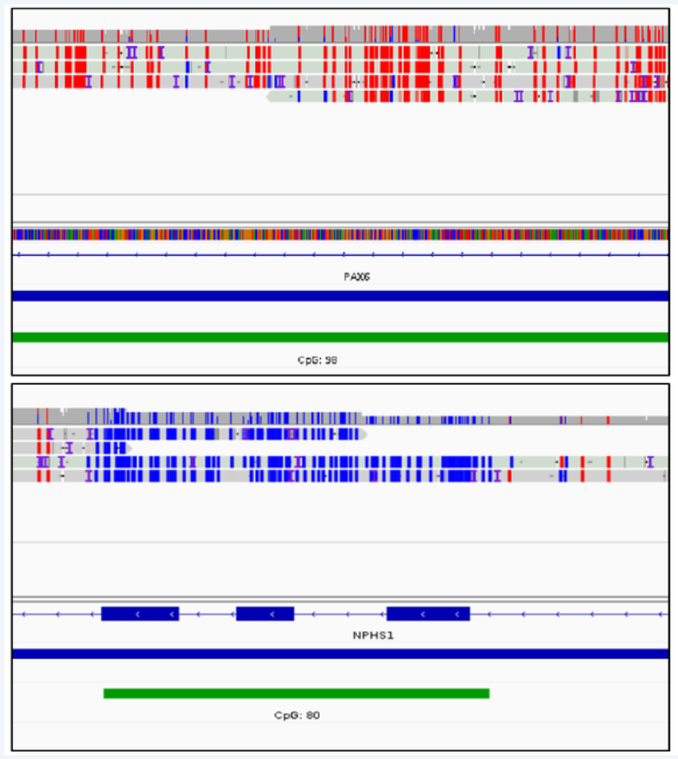
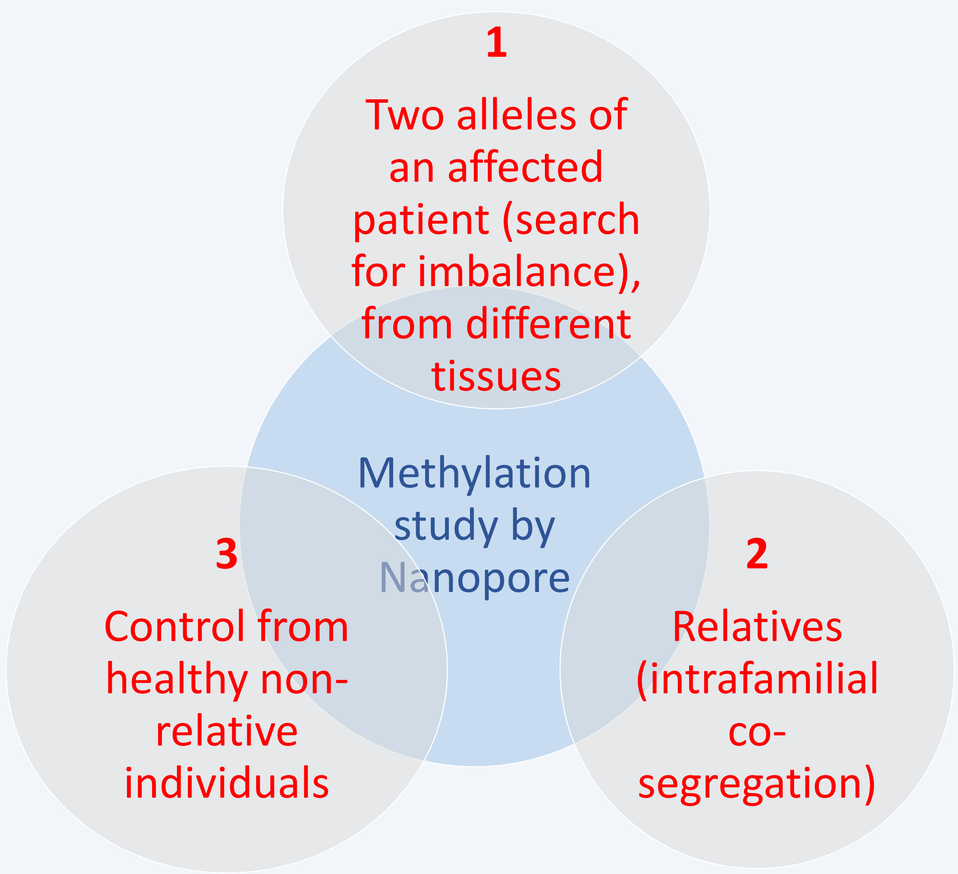


Figure 1 : Methylation profile of PAX6 and NPHS1 genes in podocytes. *Nephrin protein (NPHS1), a podocyte marker, is highly expressed in this cell type, whose expression correlates with the hypomethylation of CpG islands shown in blue. PAX6 is a gene expressed mainly in the retina and brain, and is virtually absent in kidney cells and podocytes, which explains the repression of its expression by the methylation of CpG islands shown in red.*

Method



In order to study the methylation phenomenon at the level of TMA-associated genes, we propose to use Nanopore "long read" sequencing to examine patients and families followed up at Tenon Hospital for *CFH*, *CD46*, *CFI* or even Hybrid gene TMA (***17 families*** *with* ***variable c-TMA phenotype*** *and* ***incomplete penetrance)***

- **DNA extraction** can be performed on blood and urine samples, given the tissue expression of *CFH*, *CFI* and *CD46*.
- **Nanopore sequencing** will be used to **confirm the variants** found in the exome or by panel, and at the same time to **study the methylation profile** of the c-TMA target genes.
- **Expression study** using **qPCR.** The nanopore technique could also sequence RNA directly.

Objective and prospect

The aim of the study is to determine whether the regulatory region of these genes exhibits **methylation heterogeneity**, and to try to explain some of the clinical heterogeneity of TMA by **promoter methylation abnormalities.**

Benefits for the field of nephrology:

- **Rapid molecular diagnosis** (4-5 days), providing **early guidance on treatment of c-TMA.**
- **More proactive management** and **prediction of post-transplant relapse.**
- **More personalized genetic counseling** based on the level of risk associated with the methylation profile

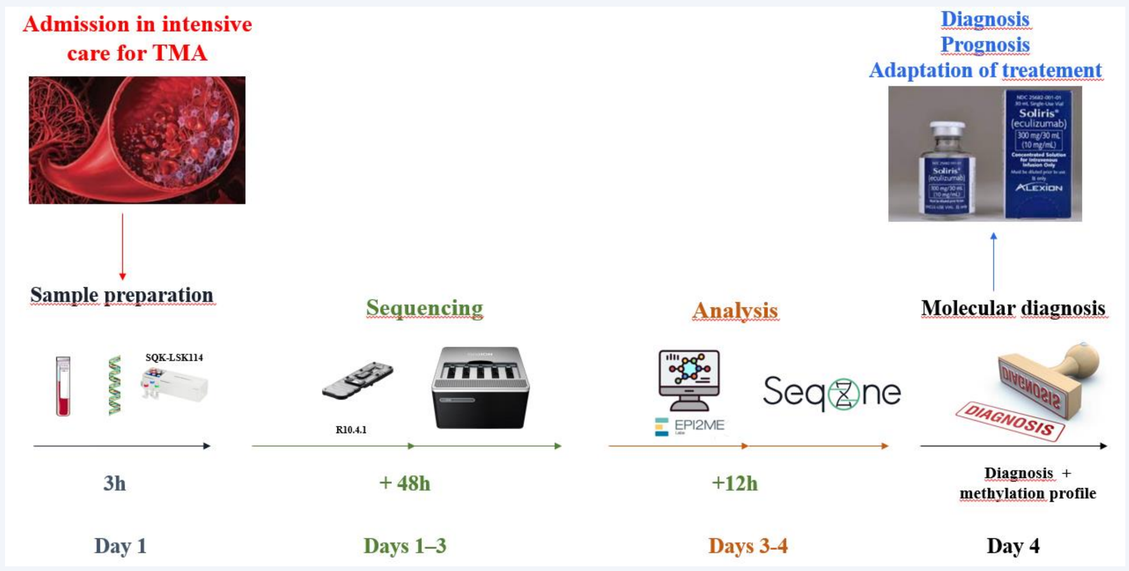


Figure 2 : Workflow for rapid diagnosis by Nanopore for TMA