GENETIC DIAGNOSIS OF INHERITED KIDNEY DISEASES BY MODELIZATION IN ZEBRAFISH

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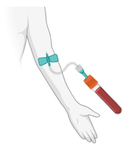
INTRODUCTION

In Europe, about 70 per 100 000 individuals suffer from inherited kidney disease [1]. However, more than 70% of cases remain undiagnosed. Patients' quality of life could be improved by accurate genetic diagnosis, which would provide a better prognosis and allow personalised treatment. Treatments for most rare diseases remain scarce due to a lack of experimental models in which candidate variants can be validated and disease mechanisms can be explored. In this context, the zebrafish (Danio rerio) has been widely used to validate candidate human disease variants and to elucidate the molecular mechanisms and pathophysiology of disease, as well as for drug discovery. Therefore, our main goal is to improve genetic diagnosis by generating zebrafish models that mimic mutations found in patients and provide an overview of the associated phenotypes.

GENERAL PLAN

1) Clinical evaluation

- Patients with CAKUT or ciliopathies



2) Blood sample

3) Exome sequencing

- NovaSeq 6000
- 2X100 Paired-End sequencing

4) Bioinformatic data processing

- Map to reference genome
- Mark duplicates
- Identify variants

Agilent Alissa

5) Variants sorting

- Interpret and prioritise variants according to the ACMG guidelines

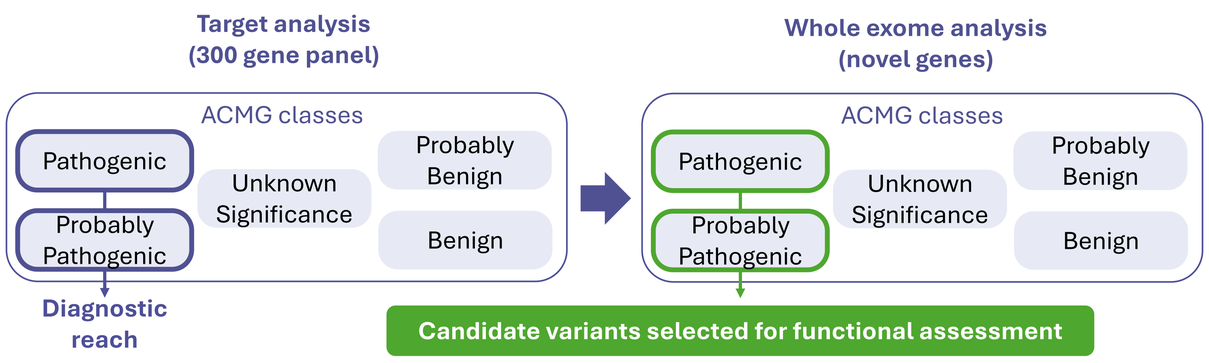
6) Assessment of pathology

- Initial assessment of candidate genes
- Stable generation of human variants

Collaborations : Pr. Bours and Dr. Jacquinet (Liège University), Pr. Mesnard (Sorbonne University), Pr. Jobst-Schwan (Erlangen University)

Variants sorting

- ACMG criteria classify variants into 5 categories (pathogenic to benign) [2]
- A panel of 300 genes is already known to be responsible for kidney abnormalities
- If the genetic analysis on the 300-gene panel does not lead to a diagnosis, we proceed to full exome analysis
- We aim to discover new candidate genes involved in kidney disease through whole exome analysis



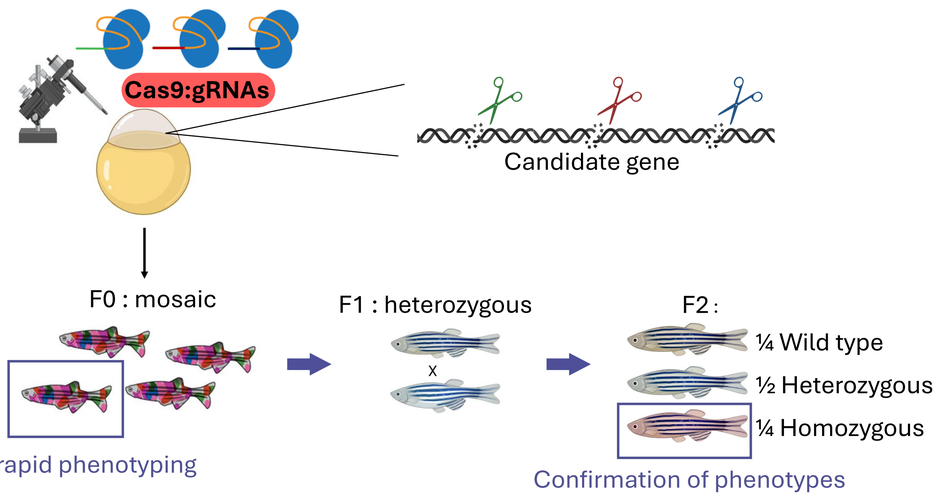
ACMG criteria [2] :

- ✓ Type and location of the mutation
- ✓ Conservation between species
- ✓ Grantham distance
- ✓ Allele frequency in the population
- ✓ In silico prediction
- ✓ Databases
- ✓ Literature
- ✓ Segregation studies

Assessment of pathology

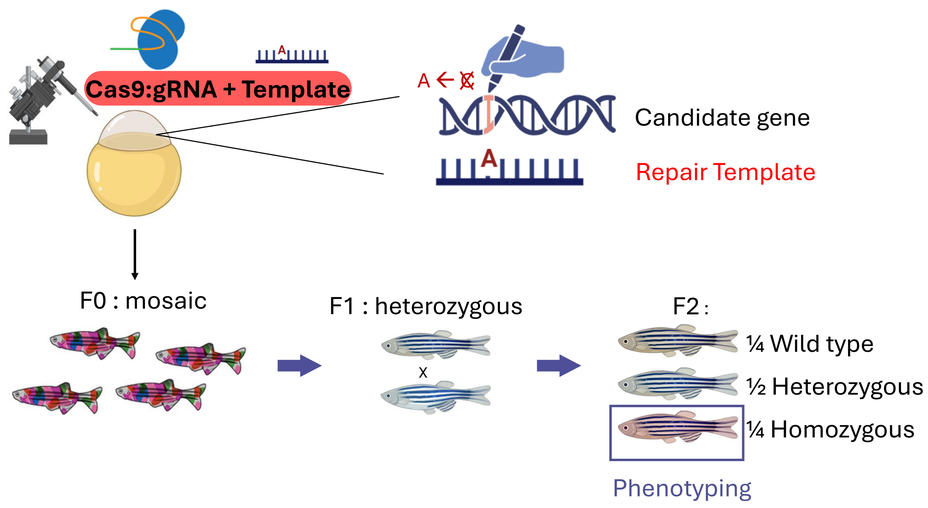
Initial assessment of candidate gene

- Rapid and moderate-throughput screening of candidate genes
- Initial assessment of pathogenicity by generation of null mutants
- Injection of multiple CRISPR/Cas9 complexes targeting the candidate

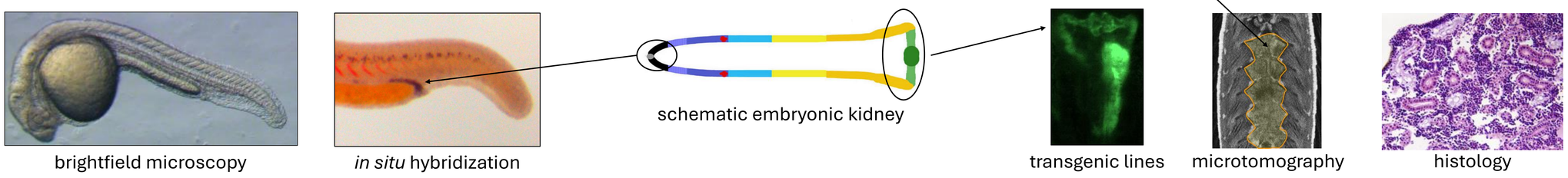


Stable generation of human variant

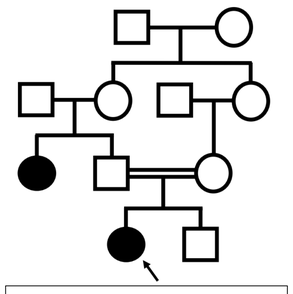
- If the initial assessment reveals phenotypes associated with kidney disease
  - ↳ Generation of a zebrafish line carrying the same mutation as found in the patient
- Injection of a CRISPR/Cas9 complex and a repair template containing the patient's mutation for a precise editing



Phenotyping



EXEMPLE OF THE WWC1 GENE



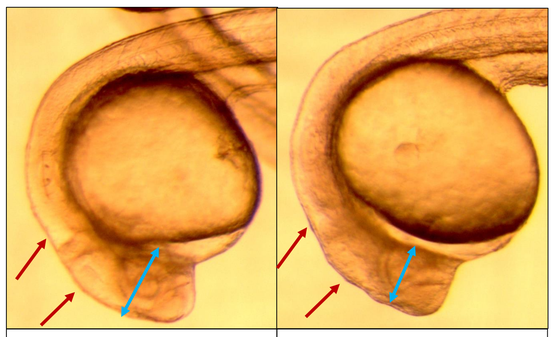
Patient with kidney and brain abnormalities

Variant :

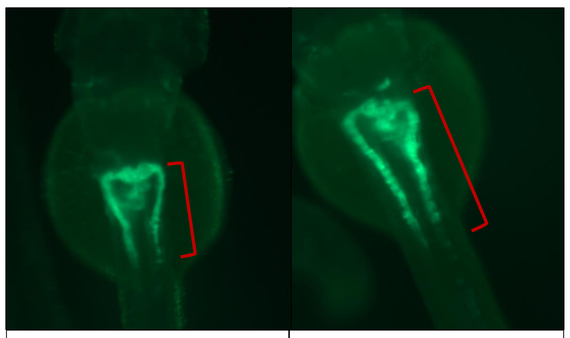
- WWC1:c.1397A>G homozygous
- Extremely low frequency in population databases
- Highly conserved amino acid
- Predicted to be deleterious by in silico programmes

(Pr. Jobst-Schwan : Erlangen University)

INITIAL ASSESSMENT IN F0 MOSAIC



Control	Knock-out FO
• 24 hpf • Thinner head and brain (blue) • Less well defined brain cavities (red)	



Control	Knock-out FO
• 55 hpf • Transgenic Tg (wt1b:eGFP) • Longer expression in the pronephros (red)	

CONCLUSION AND PERSPECTIVES

- Thanks to this strategy, we have already identified the NR6A1 gene as a new gene involved in congenital urogenital anomalies [3]
- We now want to confirm the role of WWC1 in kidney disease (F1 analysis)
- In the future, we want to extend this study to other new genes to increase the rate of patient diagnosis and discover new genes involved in kidney disease

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[1] O. Devuyst, N. V. A. M. Knoers, G. Remuzzi, et F. Schaefer, « Rare inherited kidney diseases: challenges, opportunities, and perspectives », *The Lancet*, vol. 383, nº 9931, p. 1844-1859, mai 2014, doi: 10.1016/S0140-6736(14)60659-0.

[2] S. Richards *et al.*, « Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology », *Genet. Med.*, vol. 17, nº 5, p. 405-424, mai 2015, doi: 10.1038/gim.2015.30.

[3] A. Jacquinet *et al.*, « Identification of NR6A1 as a new gene involved in congenital urogenital anomalies », présenté à 56th European Human Genetics Conference 2023, 2023. Consulté le: 16 avril 2024. [En ligne]. Disponible sur: https://orbi.uliege.be/handle/2268/313701