Gene replacement therapy in a kidney stone mouse model

Location: Boston, MA, USA
Institute: Boston Children's Hospital/Harvard medical school

Background

Urinary stone disease (USD) is prevalent and affects approximately 1:10 people during their lifetime. Here, crystals, i.e. stones, are formed in kidney. These severely affect quality of life, as stones can induce colicky pain, require hospitalisation and recurrent surgery. Currently, the treatment of USD costs approximately $10 billion a year and has been increasing. Presently, therapeutic options are limited and require lifelong adjustments in lifestyle, while the recurrence of kidney stones remains approximately 50%.

Genetic factors are a known contributor to the development of USD. Approximately 15% of hospitalised patients with USD have a monogenic defect. Particularly in congenital USD, the reabsorption of electrolytes from urine is disturbed. Various ion transporters or channels may be affected, such as the Cl^-/H^+ antiporter 5 (CLC5), which is often disturbed in Dent's type I disease. Damaging variants in the *CLC5* gene lead to proteinuria, hypercalciuria, hyperphosphaturia, and kidney stones formation. The protein is mainly expressed in the proximal tubule (PT) of the nephron and present in endosomes. Dysfunction of the endosomes in PT is associated with disturbed protein, lipid, Ca^{2+} and Ox metabolism. Indeed, mice lacking the *Clc5* gene exhibit hypercalciuria and proteinuria. Currently, there are no therapies available that target the genetic causes of USD.

Project objective

In this project, the therapeutic potential of adeno-associated viruses (AAV) will be tested for gene replacement therapy (GRT). Here, we aim to transduce the kidneys of *Clc5* deficient (*Clc5*$^{-/-}$) mice with AAVs containing the *Clc5* gene to restore its expression. Mice will be injected at a young age with one of the following viruses: 1) CAG-*Clc5,* 2) *Sglt2-tdTomato*, and 3) *Sglt2-Clc5*. These viruses allow us to determine I) proximal tubule specificity and II) rescue potential of the transgene. Urinary calcium and protein excretion will be monitored to determine the rescue by AAV-mediated transduction on a physiological level. At the end of the experiment, mice will be euthanized, and kidneys will be collected. We will then determine mRNA and protein expression of the transgenes.

Main research question:

- Does retro-orbital administration of AAV containing the *Clc5* transgene result in GRT on a cellular and physiological level in Clc5$^{-/-}$ mice?

Aims

- Assess urinary calcium and protein excretion in *Clc5*$^{-/-}$ mice post AAV-injections.
- Determine *Clc5* transgene expression in kidneys and other tissues on mRNA/protein level.

If time permits also the following can be included:

- Establish an in vitro proximal tubule model to assess CLC5 function.

The lab

We offer the possibility to perform and present translational research in a professional, multicultural, and highly motivating working environment with about 15 colleagues in a well-equipped department. The Nephrology department is located in the **Boston Children's Hospital/Harvard Medical School, Boston, MA, USA.** The lab is led by Prof. Friedhelm Hildebrandt, a geneticist who has found ~40 genes responsible for various monogenic kidney diseases. You will work under the supervision of Gijs Franken, a former PhD

candidate of the Department of Physiology, Radboudumc, Nijmegen. You will perform your internship under his daily supervision and will get acquainted with techniques, such as cell culture, viral transduction, lab animal handling, real time quantitative PCR, immunohistochemistry, and western blot.
You will be part of an international group of postdocs from various countries, including Germany, India, Japan, and Israel. During the week, you will have various personal and group meetings to discuss the progress of your and other projects.

What we seek

We are searching for a highly motivated **Master student** with a background in biology/biomedical sciences who wants to gain experience abroad. The student should already have wet-lab experience, but does not necessarily need to have experience with every technique mentioned above. The student will work with animals, i.e. husbandry, injections and dissections. Experience with lab animals is not necessary (although is a plus), but willingness to learn is essential. Possession of the Article 9, according to Dutch law to work with lab animals, is not required. The internship is for a **minimum of 10 months** with a preference for a year. The position is readily available, but it must be kept in mind that student will have to apply for a US visa, which takes ~4-5 months. The supervisor will also support the student to gather funds.

Are you interested in the position? Please provide a CV and motivation letter and send to gijs.franken@childrens.harvard.edu

Other information

Information on the lab
PI: https://www.childrenshospital.org/directory/friedhelm-hildebrandt
Daily supervisor: linkedin.com/in/gijs-franken-2169528a

Literature
Principal of AAV-mediated GRT: https://pubmed.ncbi.nlm.nih.gov/37556557/
Background on Clc5 deficient mice: https://pubmed.ncbi.nlm.nih.gov/12548389/

Funding
Funding option 1: https://nvbmb.kncv.nl/grants/nbs-travel-grants-for-internships/nbs-and-febs-travel-grants-for-internships
Funding option 2:
https://nierstichting.nl/documents/120/infosheet_student_researcher_grant_2021_dd_5feb21.pdf
Funding option 3 (BMS students/MMD students only):
https://www.radboudumc.nl/en/education/international-office/outgoing-students/more/for-students-biomedical-sciences/for-students-biomedical-sciences#68034