In-depth study of biochemical mechanisms in genetic disorders of lysine metabolism: Generation and characterisation of human cellular models of pyridoxine-dependent epilepsy and glutaric aciduria type 1

Imke M.E. Schuurmans[1,2], Alejandro Garanto[1,2,3], Karlien L.M. Coene[2,4], Nael Nadif Kasri[3,5] and Clara D.M. van Karnebeek[1,2,6]

[1]Department of Pediatrics, [2] Radboud Institute for Molecular Life Sciences, [3]Department of Human Genetics, [4]Translational Metabolic Laboratory, Department of Laboratory Medicine, [5]Donders Institute for Brain, Cognition and Behaviour and [6]Amalia Children's Hospital, Radboud university medical center, Nijmegen (The Netherlands)

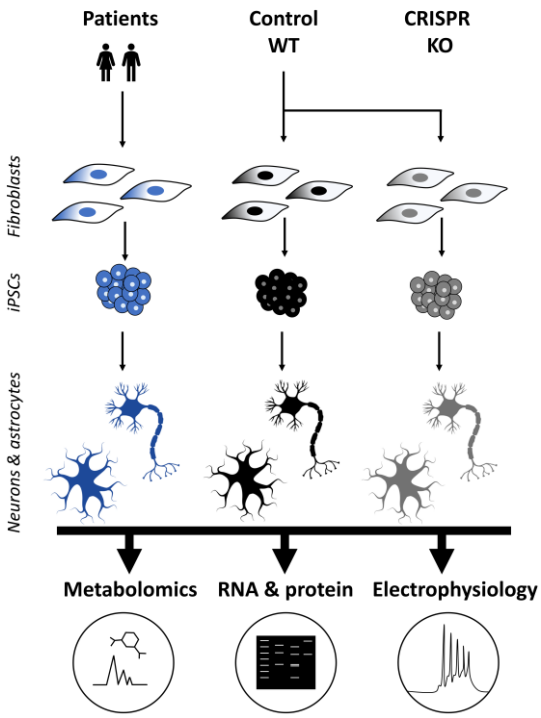
Background

- PDE-ALDH7A1 and GA1 are two rare neurometabolic disorders of lysine metabolism
- Caused by biallelic mutations in *ALDH7A1* (PDE) and *GCDH* (GA1) resulting in accumulation of neurotoxic metabolites leading to severe and potential fatal neurological sequalae
- No human cellular models for PDE-ALDH7A1 and GA1 available
- Mechanism of neurotoxicity is still unclear and therefore no effective treatments for both diseases are available

Aim

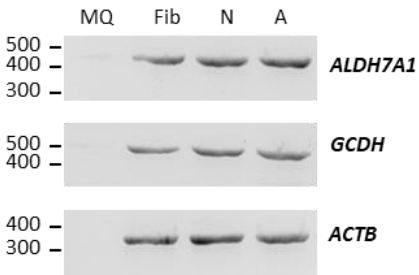
Generate human cellular models for PDE-ALDH7A1 and GA1 to perform an in-depth molecular, electrophysiological and metabolomic characterization

Methodology



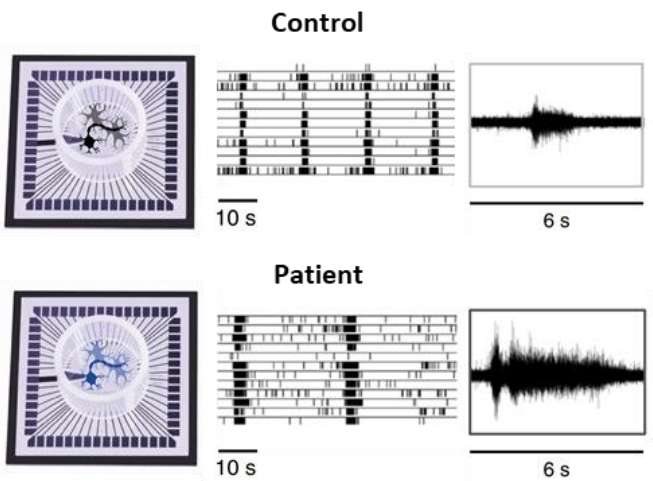
Patient-derived fibroblasts will be reprogrammed into induced pluripotent stem cells (iPSCs). Patient, control (WT) and CRISPR-generated knock-out (KO) iPSC lines will be differentiated towards neurons and astrocytes and cultured on micro-electrode arrays (MEAs) for electrophysiology analysis. In addition, cell extracts and medium of both the fibroblasts and neuron and astrocyte co-cultures will be collected for targeted and untargeted metabolomic analysis. Molecular techniques (Western blot and qPCR) will allow the study at RNA and protein level.

Molecular characterization



Preliminary results confirmed that both *ALDH7A1* and *GCDH* are expressed in human fibroblasts (Fib), neurons (N) and astrocytes (A). This highlights the utility of fibroblasts, neurons and astrocytes as models to study these diseases.

Functional characterization



Micro-electrode arrays will be used to analyse the network activity of neuronal and astrocyte co-cultures. By combining various parameters such as the spontaneous electrical activity rate and burst rate, we will define a disease specific electrophysiological phenotype for PDE-ALDH7A1 and GA1. Image adapted from Frega et. al 2019.

Conclusion

By in-depth molecular, electrophysiological and metabolomic characterization of fibroblast cells and neuronal and astrocyte co-cultures, we will be able to define novel robust cellular PDE-ALDH7A1 and GA1 models that will serve to shed further light on disease mechanisms and test potential therapeutic strategies.

Support

EUROPEAN JOINT PROGRAMME RARE DISEASES

Institute for Molecular Life Sciences Radboudumc

Human Genetics Nijmegen

Amalia kinderziekenhuis Radboudumc

Imke.schuurmans@radboudumc.nl