Drug discovery for GNAO1 mediated disease, elucidating the mechanisms underlying a spectrum of neurodevelopmental disorders.

AIM

To identifying druggable pathways in order to restore normal GNAO1 function. To this end we will generate in vitro neuronal models to study the alterations in patient specific GNAO1 mutation carrying cells.

Background:

Development of the nervous system is a very delicate process. Cell signaling and cell-cell interactions guide the development and positioning of the neuronal circuits. Once the right interactions are established, maintenance of this structure is essential. Regulation of neurotransmitter release is an important player in this process. The GNAO1 gene encodes the alpha-subunit of the guanine nucleotide binding protein Gao. This protein is the most abundant Gα in the central nervous system. In the brain it comprises up to 1% of total membrane protein, with high expression in cerebellum, hippocampus and striatum. It interacts with important G-protein coupled receptors (GPCRs) like GABA, adenosine and dopamine D2 receptors. All these receptors play an important role in neurodevelopment, regulation of movement and neurotransmitter release.

In view of the important role of Gao it is not surprising that mutations in GNAO1 result in neurological symptoms. Surprisingly, the spectrum of GNAO1 mutations is very wide, ranging from early onset childhood epilepsy (Ohtahara syndrome, early infantile epileptic encephalopathy 17 (EIEE17)) to movement disorders with or without epilepsy (NEDIM) (Saitsu et al., 2016). This broad phenotype can possibly be explained by the nature of the different GNAO1 mutations. In Ohtahara syndrome the mutations are proposed to be lossof-function (LOF) mutations and in NEDIM cases the mutations could result in a gain of function (GOF) (see review by Feng et al, 2018, Neurobiology of disease 131-141). Dysregulation of cAMP signaling, neurotransmitter release and neurodevelopment are proposed to be the determinants of the disease spectrum. However, human models that recapitulate the patients' phenotypes in a dish are required to assess the contribution of each determinant to the disorder.

Approach

1) Generate iPSC lines from GNAO1 patients and correct the mutation with CRISPR/Cas9 technology to generate isogenic controls.
2) Differentiate the lines to a neuronal lineage, aiming at cerebral and cerebellar differentiation in 2D and 3D cultures (organoids).
3) Analyze gene expression patterns of 2D and 3D cultures by RNA-seq and pathway analysis.
4) Analyze differences in neural development between GNAO1 and isogenic control organoids.
5) Study the network activity of GNAO1 neurons using calcium imaging and multielectrode arrays (MEA).
6) Identify GNAO1 mutation linked phenotypes that can be used for drug screening.
7) Develop drug screening assay
8) Establish collaborations with academic and pharma labs for drug screening

The team: Prof. Frank Baas, Prof. Arn van den Maagdenberg, Dr Harald Mikkers
At the LUMC, the laboratories (Baas and van den Maagdenberg) have been working on different aspects of neurodevelopment for many years. Frank Baas' group has identified genes for neurodevelopmental and movement disorders, including GNAO1 mutations and has expertise in drug development (www.complementpharma.com) Arn van den Maagdenberg's group studies migraine and epilepsy with focus on mechanisms of signal transduction and electrophysiology. Harald Mikkers is head of the LUMC iPSC hotel and his group has vast experience in the generation and genetic modification of iPSCs and neuronal differentiation thereof.

Current status of the project
iPSC lines have been generated from 4 GNAO1 patients with neurodevelopmental and movement disorders. A research technician has been trained for iPSC culture and differentiation. RNA expression profiling, MEA and calcium imaging are standard techniques at the Baas and van den Maagdenburg labs.

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