Summer school 2021

Orphan disease:
A 4-year-old boy with an unknown muscle disease

Photo: The patient with his father (right) and the medical doctors/(clinician-)scientists (left). Copyright Ivar Pel, 2018.

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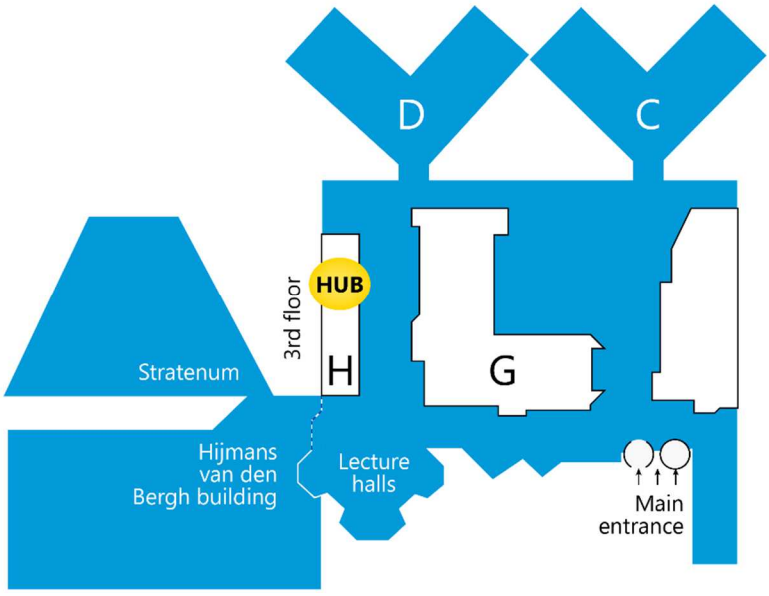
Preface

Welcome to the first edition of the Bachelor Research Hub Summer school, taking place in the newly equipped laboratory positioned in the heart of the University Medical Centre Utrecht. During the 2-week period you will be performing an authentic and real-world actual research under supervision of professionally trained teacher-researchers. You will experience a "crash-course" in the scientific world: from thinking about the topic, investigating the current literature, and writing a short project proposal, to conducting the experimental part of the research, and finally presenting and discussing your data. Sharing your findings and knowledge is always an important part of the research, therefore it is useful to get acquainted with it and practice it at the very start of your scientific careers.

The topic of the Summer school will revolve around a four-year-old boy with an unknown (orphan) disease. The boy suffers from muscle weakness and inadequate muscle reflexes with signs of floppy infant syndrome. This hinders his every-day life as well as his development due to the tiredness and frequent need for a wheelchair. While many diseases have been ruled out by extensive medical testing, no diagnosis has yet been established and the cause of the patient's disease remains unknown. The first insights towards unravelling the mechanisms behind the disease were hinted via whole-genome sequencing where a previously unrecognized single-nucleotide change in the patient's genomic DNA was revealed. During the course, you will continue investigating this disease by incorporating your own ideas and choosing your own research paths. As a starting point, see the brief proposal below.

We wish to welcome you to our laboratory where, with a support of many supervisors, you can get a glimpse of the real scientific world. Happy researching!



Location: Bachelor Research Hub (H03.201), UMC Utrecht (Heidelberglaan 100, 3584 CX Utrecht)

With kind regards,

Niels Bovenschen, Toine ten Broeke, Sandra Crnko, Emma Pijnappel and Jan Meeldijk

Literature

The following articles and videos will give you an insight into the topic and techniques you will be using during the Summer school.

1. **How a four-year-old boy connects healthcare, biomedical research and undergraduate education**
   Rijkent H Drost, Wim J A G Dictus, Berent J Prakken, Niels Bovenschen
   Nat Biotechnol. 2019 Sep;37(9):1092-1095.
   https://pubmed.ncbi.nlm.nih.gov/31485047/

2. **Bachelor Research Hub**
   https://www.youtube.com/watch?v=PmBeLLvpXHw

3. **Western blot tutorial**
   https://www.youtube.com/watch?v=CEEekahiqMo

4. **Cell culture tutorial**
   https://www.thermofisher.com/nl/en/home/references/gibco-cell-culture-basics/introduction-to-cell-culture.html

5. **Flow cytometry tutorial**
   https://www.youtube.com/watch?v=sfWWxFBltpQ

Schedule

Week July 5th-9th:

Monday:

1.	Gathering at the main entrance of UMC Utrecht	08:55-09:00h	Everyone
2.	Introduction to the Summer school	09:00-09:15h	N. Bovenschen
3.	Introduction of the topic	09:15-10:00h	N. Bovenschen
4.	Hypothesis formulation, planning and preparations	10:15-17:00h	Students

Tuesday:

1.	Lab journal	09:00-09:30h	T. ten Broeke
2.	Discussion of the hypothesis and planned lab work	09:30-10:00h	Everyone
3.	Lab work*	10:15-17:00h	Students

Wednesday:

1.	Lab work	09:00-17:00h	Students

Thursday:

1.	Lab work	09:00-17:00h	Students

Friday:

1.	Recap results (short ppt) + Planning for the next week	09:00-09:45h	Everyone
2.	Lab work	10:00-17:00h	Students

Week July 12th-16th:

Monday:

1.	Lab work	09:00-17:00h	Students

Tuesday:

1.	Lab work	09:00-17:00h	Students

Wednesday:

1.	Lab work	09:00-17:00h	Students

Thursday:

1.	Lab work	09:00-17:00h	Students

Friday:

1.	Presentation preparation	09:00-15:00h	Students
2.	Final presentation (30min+discussion)	15:15-16:00h	Students
3.	Closing remarks	16:00-17:00h	N. Bovenschen

**Lab work includes: molecular biology, cell culture, Western blot, Flow cytometry, etc*

Proposal

Investigating the cause of congenital muscle weakness by studying phosphorylation of Pyk2 and FAK

Abstract

A *de novo* point mutation in the protein Pcdh-γ, V694A, has been found in a patient suffering from muscle weakness. Pyk2 and FAK are kinases that interact with Pcdh-γ and are involved in intracellular signaling related to neuronal function. Mutation could potentially impair the binding of Pcdh-γ to Pyk2 and/or FAK, thereby increasing phosphorylation of these kinases and causing neuronal defects.

Introduction

A patient (age 4) has suffered from severe muscle weakness since birth, experiencing reduced muscle tonus and delayed development. After birth a small visage, disproportionally large extremities, *pectus excavatum*, heart murmurs, a small palate and low birth weight were noted. Physiotherapy and supplementary feeding improved the patient's condition, resulting in him being able to sit and walk on his own.

Multiple tests were used to determine the cause of this phenotype. MRI-scans of the brain, metabolic testing and genomic tests specific for Angelman syndrome and Williams syndrome showed no abnormalities. Analysis of a muscle biopsy taken at the age of two revealed a significant increase in type C2 muscle fibers, a sign of muscle development and/or regeneration. The muscle fibers were overall smaller and more spaced out, while in certain regions myofibril destruction and loss of mitochondria were noted.

After previous testing being inconclusive, Whole Exome Sequencing (WES) was performed. This resulted in the detection of one possibly significant mutation in the gene PCDHγB3, located on chromosome 5. It concerns a T2081C de novo mutation resulting in the amino acid substitution V694A. The PCDHγB3 gene encodes for protocadherin gamma B3, which is part of the protocadherin gamma gene cluster. Protocadherin gamma is a transmembrane protein that is part of the cadherin superfamily found in multiple species, for example Rhesus macaques (*Macaca mulatta*)[1]. It is predominantly located in synapses[2], although it is also present in other tissues such as skeletal muscle[3]. The extracellular domain of this protein binds to other protocadherins, while the intracellular domain prevents autophosphorylation of the kinases FAK and Pyk2 (figure 1)[4]. Protocadherin gamma is believed to play a critical role in the formation of synaptic connections. This notion is supported by reduced synapse formation and increased synapse apoptosis in mice lacking this gene cluster2. Inhibition of FAK and Pyk2 auto-phosphorylation also stimulates development of neural dendrites.[4] This makes the mutant PCDHγB3 allele a likely contributor to the phenotype of our patient, and thus an interesting target for further research.

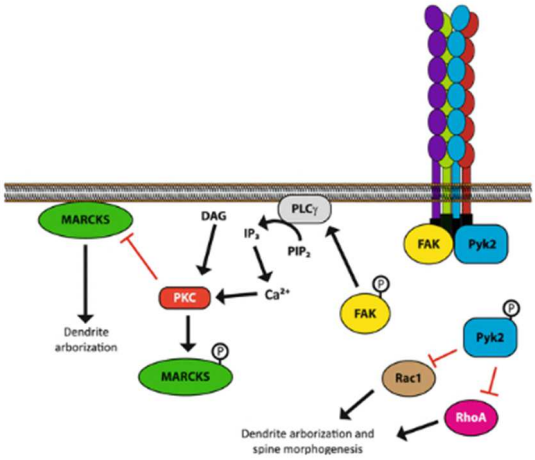


Fig. 1: *Downstream signaling cascade of PCDHγB3: FAK and Pyk2 directly bind to PCDHγB3 intracellularly[4].*

Aim of the study

The aim of the study is therefore to determine the influence of V694A mutated PCDHγB3 on the amount of phosphorylation of downstream effector proteins FAK and Pyk2 compared to the wild type protein.

Methods

First, both the wild type PCDHγB3 gene and mutant (V694A) PCDHγB3 gene will be multiplied by infecting *E. coli* bacteria with the corresponding plasmids. From these colonies plasmids containing the wild type/mutant PCDHγB3 gene are isolated. Then, the plasmids are transiently transfected into separate HeLa cells. The protocadherins will be expressed after 24 hours, so the HeLa cells will be incubated for 24 hours. The HeLa cells will then be lysed using SDS-buffer, after which the isolated proteins will be run on a SDS-PAGE gel. Thereafter, the protein solution will be transferred to a Western Blot to identify FAK and Pyk2. This is done with fluorescently labelled monoclonal antibodies specifically binding to the phosphorylated proteins and antibodies binding to both the phosphorylated and unphosphorylated versions. Based on the ratio between both bands, the rate of phosphorylation can be determined semi-quantitatively .

Anticipated results

The rate of phosphorylation of the downstream effector proteins FAK and Pyk2 is measured for both cultures. The expectation is that the amount of phosphorylation of both FAK and Pyk2 will be higher in the HeLa cells transfected with mutant PCDHγB3 (V694A) compared to the HeLa cells transfected with wild type PCDHγB3. This indicates that the activity of mutant PCDHγB3 is lower than the activity of wild type PCDHγB3.

Discussion:

The assumption is made that PCDHγB3 will be absent in the wild type HeLa cells. Research of Han *et al*[5] showed transfection of HeLa cells with the PCDHγB3 gene. However, it is not proven that HeLa cells do not contain the PCDHγB3 gene. Absence of this protein in the cell line is important, as native PCDHγB3 proteins will also inhibit autophosphorylation, influencing the results. Secondly, it is important that downstream proteins FAK and Pyk2 are present in the HeLa-cells, as they need to be phosphorylated by the PCDHγB3 protein. Several studies do indicate that both downstream proteins are present in HeLa-cells[6,7].

References

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