Tuesday, August 29, 2023

International PhD PPU-IMAGINE Proposal

Call 2023/2024

PhD PROPOSAL IDENTIFICATION

PhD Project title	Understanding the physiopathology of CERebellar DEVelopmental defects using human cellular models and zebrafish
Project Acronym	CerDev
Project Keywords	Cerebellum, Malformation, iPSC, Organoid, Zebrafish

LABORATORY PRESENTATION

Laboratory Team Name	Developmental Brain Disorders Laboratory
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PhD PROPOSAL

PhD Supervisor full name	Vincent Cantagrel
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Number of ongoing PhD students supervised by the Supervisor	1

PhD Proposal abstract (1000 characters maximum)

Developmental defects of the cerebellum are associated with motor but also non-motor disorders such as intellectual disability and autism. About half of patients with these defects don't receive an etiological diagnosis. Additionally, the disease and developmental mechanisms involved are rarely understood when a causative mutation is identified. In our laboratory, we combine human genetics approaches with disease modeling to better understand the genetic causes and underlying pathological mechanisms. We recently identified several patients with a peculiar hindbrain malformation and/or cerebellar symptoms and de novo loss-of-function mutations in new disease gene. The goal of this project is to use human induced

pluripotent stem cells-derived models and zebrafish to characterize a new genetic disorder and uncover new cellular and molecular mechanisms involved in human hindbrain development.

PhD Proposal (10 000 characters maximum)

UNDERSTANDING THE PHYSIOPATHOLOGY OF CEREBELLAR DEVELOPMENTAL DEFECTS USING HUMAN CELLULAR MODELS AND ZEBRAFISH

PROJECT DESCRIPTION

Developmental brain disorders (DBD) encompass a highly heterogeneous group of diseases characterized by impairments in cognition, communication, behavior or motor functioning as a result of atypical brain development. The clinical assessment, including brain MRI of numerous DBD cases suggests that the cerebellar development can be specially affected. The human cerebellum is a brain region known to play a key role in motor development but also, a less understood one, in cognition (**1**). Currently, for almost half of the patients with DBD of suspected genetic origin, there is no genetic diagnosis. Additionally, the disease and developmental mechanisms involved are rarely understood when a causative mutation is identified. In our laboratory, we combine human genetics approaches with disease modelling using human induced Pluripotent Stem Cells (hiPSC)-derived models and zebrafish to better understand the genetic causes and underlying pathological mechanisms of DBD (**2, 3**). Recently identified gene defects suggest that haploinsufficiency of key genes involved in cerebellar development is a cause of DBD with broad clinical consequences including intellectual disability and autism. In our cohort of DBD patients, we have identified multiple cases with heterozygous loss-of-function mutations in genes known or suspected to play a key role in human neurogenesis and latter cerebellar developmental steps.

One of these patients is affected with psychomotor developmental defects and a rare and peculiar malformation of the pons and cerebellum called pontine tegmental cap dysplasia (PTCD). It is characterized by an abnormal shape of the brainstem and cerebellum on MRI. However, so far, no genetic or environmental causes have been identified for this defect. Diffusion tensor imaging in this patient detected white matter fiber tracts disorganization in the hindbrain, as previously observed in other cases with PTCD (**4**). Genetic investigation identified a de novo mutation in a gene not previously associated with a Mendelian disorder, highly expressed during cerebellar development and playing a role in axon guidance and transcription regulation. Investigation of other cohorts with DBD identified at least one additional case with de novo loss-of-function mutation and similar symptoms associating motor-coordination defects with cognitive impairments. Among others, these observations support the identification of a new DBD syndrome with disrupted cerebellar development and caused by a new genetic defect.

RESEARCH PROGRAM

1- Development of cellular models using patient-derived induced pluripotent stem cells (iPSCs) and genome base editing

The cellular reprogramming of cell line from the index patient with PTCD is ongoing at the stem cell core facility of the Imagine Institute. We have established adenosine and cytosine base editing technics in the lab as well as other genome editing methods. These technics will be used to correct patient's mutation and also to create cell lines with the other loss-of-function mutation. The goal is to obtain mutant cell lines with their respective isogenic controls.

2- Differentiation of iPSCs into cellular models for cerebellar development to study the impact of the mutations at the protein, transcriptomic and neuronal levels

The PhD student will use 2D and 3D cellular models for cerebellar development. This work is based on recently published protocols for the study of cerebellar granule cells (**5**) and Purkinje cells (**6**). In parallel, 3D cerebellar organoids will be cultured based on a protocol that we have fully established and optimized in the lab. First, the consequence of the mutations will be studied at the transcript and protein levels to detect an alternative transcript-specific impact and a decrease of protein expression levels. Then, cellular differentiation will be studied with emphasis on neurites and axon developments. Finally, the candidate will study the consequences of the mutation at the transcriptomic level during differentiation of 2D and 3D cell models.

3- In vivo consequences on cerebellar development using Zebrafish

Preliminary work identified the fish orthologous gene and characterized its expression. Genome editing

will be used to create loss-of-function mutant/crispants at the heterozygous and homozygous states. Hindbrain structural defects will be investigated (3) as well as transcriptomic deregulation during development.

This project will be dedicated to the investigation of models for a new DBD syndrome. This study can identify the first genetic factor causing PTCD and it can uncover new molecular mechanisms involved in human hindbrain development.

REFERENCES

1- Haldipur P, Millen KJ. **What cerebellar malformations tell us about cerebellar development.** Neurosci Lett. 2019 Jan 1;688:14-25. doi: 10.1016/j.neulet.2018.05.032. PMID: 29802918.

2- Ucuncu E, Rajamani K, Wilson MSC, Medina-Cano D, Altin N, David P, Barcia G, Lefort N, Banal C, Vasilache-Dangles MT, Pitelet G, Lorino E, Rabasse N, Bieth E, Zaki MS, Topcu M, Sonmez FM, Musaev D, Stanley V, Bole-Feysot C, Nitschke P, Munnich A, Bahi-Buisson N, Fossoud C, Giuliano F, Colleaux L, Burglen L, Gleeson JG, Boddaert N, Saiardi A, Cantagrel V. **MINPP1 prevents intracellular accumulation of the chelator inositol hexakisphosphate and is mutated in Pontocerebellar Hypoplasia**. Nat Commun. 2020 Nov 30;11(1):6087. doi: 10.1038/s41467-020-19919-y. PMID: 33257696

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4- Engle EC. **Human genetic disorders of axon guidance**. Cold Spring Harb Perspect Biol. 2010 Mar;2(3):a001784. doi: 10.1101/cshperspect.a001784. PMID: 20300212

5- Behesti H, Kocabas A, Buchholz DE, Carroll TS, Hatten ME. **Altered temporal sequence of transcriptional regulators in the generation of human cerebellar granule cells**. Elife. 2021 Nov 29;10:e67074. doi: 10.7554/eLife.67074.PMID: 34842137

6- Buchholz DE, Carroll TS, Kocabas A, Zhu X, Behesti H, Faust PL, Stalbow L, Fang Y, Hatten ME. **Novel genetic features of human and mouse Purkinje cell differentiation defined by comparative transcriptomics.** Proc Natl Acad Sci U S A. 2020 Jun 30;117(26):15085-15095. doi: 10.1073/pnas.2000102117. PMID: 32546527

Laboratory's best 5 publications

- Recessive PRDM13 mutations cause severe brainstem dysfunction with perinatal lethality, cerebellar hypoplasia and disrupt Purkinje cell differentiation. Coolen M, Altin N, Rajamani K, (…), Cantagrel V*.
Am J Hum Genet. 2022 May 5;109(5):909-927.

- MINPP1 prevents intracellular accumulation of the chelator inositol hexakisphosphate and is mutated in Pontocerebellar Hypoplasia. Ucuncu E, Rajamani K, (…), Saiardi A, Cantagrel V*.
Nat Commun. 2020 Nov 30;11(1):6087.

- High N-glycan multiplicity is critical for neuronal adhesion and sensitizes the developing cerebellum to N-glycosylation defect. Medina-Cano D, Ucuncu E, Nguyen LS, Nicouleau M, Lipecka J, Bizot JC, Thiel C, Foulquier F, Lefort N, Faivre-Sarrailh C, Colleaux L, Guerrera IC, Cantagrel V*.
Elife. 2018 Oct 12;7.

- De novo mutation screening in childhood-onset cerebellar atrophy identifies gain-of-function mutations in the CACNA1G calcium channel gene. Chemin J, Siquier K, (…), Lory P*, Cantagrel V*.
Brain. 2018 Jul 1;141(7):1998-2013.

- Biallelic mutations in SNX14 cause a syndromic form of cerebellar atrophy and lysosome-autophagosome dysfunction. Akizu N, Cantagrel V, (…), Gleeson JG.
Nat Genet. 2015 May;47(5):528-34.

Expected profile of the candidate

We are looking for a highly motivated candidate with passion for science and a solid background in developmental biology, genetics, cellular and molecular biology and/or neurobiology. The ideal candidate

has previous laboratory experience in basic molecular and cellular biology techniques and in cell culture. The candidate should have excellent communication skills and should be a good team player.