PASTEUR PARIS UNIVERSITE (PPU) INTERNATIONAL DOCTORAL PROGRAM 2020

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PROJECT

- **FILE #02**
- **ACRONYM:** Cb-Dev
- **TITLE:** Genetic and pathological mechanisms involved in developmental defects of the cerebellum

LABORATORY

- **SURNAME, FIRST NAME:** Cantagrel, Vincent
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- **DOCTORAL SCHOOL:** BioSPC
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LABORATORY PRESENTATION AND RESEARCH TOPICS

- **SUPERVISOR HDR:** Cantagrel, Vincent, vincent.cantagrel@inserm.fr
- **SPECIFY THE TEAM NAME:** Developmental brain disorders laboratory
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DESCRIPTION OF THE PROPOSED PROJECT

- **KEYWORDS:** Cerebellum, Cognitive and motor defects, development, zebrafish, iPSCs.

- **ABSTRACT**

This project aims to decipher the molecular defects underlying pediatric neurological disorders affecting the cerebellum structure and function with consequences on psychomotor development. This project will focus more specifically on a disease called pontocerebellar hypoplasia (PCH). Affected children have a smaller cerebellum (hypoplasia) with a decreased volume of the ventral pons, a part of the brainstem. The clinical features include a very limited motor and cognitive development, swallowing and feeding difficulties along with limb spasticity. This condition is extremely severe usually lethal within the first 10 years of life. The genetic bases are only partially known, there is no treatment available and clear hypothesis are lacking about its pathogenesis. The leading hypothesis is an excess of cell death affecting neural progenitors and neurons during cerebellum development. Several pathways have been identified including RNA processing and degradation, protein translation regulation and inositol phosphate metabolism. Recently, we also identified an additional mechanism involving a defect in the transcriptional regulation of neuronal subtype differentiation. The study of this specific defect could give us a new entry point into an epigenetic network critical for proper cerebellar development.

- **DESCRIPTION OF THE PROJECT**

The human cerebellum has a well-known function in motor coordination but a new role in regulating cognitive, emotional and social behavior is emerging. Its development is disrupted in a large number of neurological disorders [1] with severe consequences on psychomotor development. Our lab aims to decipher the molecular defects underlying these pediatric

diseases [2-6]. This project will focus more specifically on pontocerebellar hypoplasia (PCH), which is a group of diseases that are characterized by hypoplasia of the cerebellum and pons, and often fatal within the first years of life. The clinical features include a very limited motor and cognitive development, swallowing and feeding difficulties along with limbs spasticity. The treatments for these disorders are only symptomatic and only half of the patients have a genetic diagnosis suggesting that new genetic causes remain to be identified. Clear hypotheses are lacking about its pathogenesis. Currently, an excess of cell death affecting neural progenitors and neurons mostly during cerebellum and pons development is suspected. Various pathways can be affected such as the tRNA splicing machinery, the RNA exosome complex, the purine nucleotide metabolism or vesicular trafficking. Recently, the investigation of undiagnosed severe PCH cases allowed us to identify mutated genes belonging to pathways not expected to be associated with this disease. These pathways are critical for neuronal differentiation and include (i) the inositol phosphates metabolism and (ii) the early specification of hindbrain neuronal subtypes.

Goals of this study:

Aim 1: **Identifying new molecular causes of PCH** *using high-throughput sequencing techniques (NGS).* A cohort of patients from Necker hospital is currently being genetically characterized using high-throughput sequencing including whole genome sequencing. Additionally, several new candidate genes have been recently identified in our genetic screen. Validation will be possible through the investigation of independent cohorts for variants in the same genes. This work includes the use of the Imagine home-made software (i.e. Polyweb) for whole exome and whole genome analysis as well as exploration of databases to prioritize candidate variants and identify additional mutations with similar clinical condition. This aim could identify mutations in additional genes belonging to the newly identified pathways involved in PCH.

Aim 2: Decipher the physiopathology mechanisms using an available animal model of PCH. We will use zebrafish to reproduce mutations identified in new PCH genes such as *PRDM13*. This gene encodes a transcriptional repressor defined by a histone methyltransferases-related domain. Its role has been only partially described. Indeed, it was shown to participate in the early specification of discrete neuronal subtypes [7] but it has not yet been implicated in cerebellum development. The zebrafish present many advantages such as a good conservation of the cerebellar organization, a fast brain development and multiple options and tools for genetic engineering and brain investigation [8]. We will characterize *Prdm13* expression in the fish and the impact of this mutation on the growth, organization and neuronal composition of the cerebellum.

Aim 3: **Study patient-derived or genome edited iPSCs to model the disease in human cells.** Using genome editing, patient's *PRDM13* mutation has been recreated in control induced pluripotent stem cells (iPSCs). Using these cells, we can apply a 3D cell culture protocol that

recapitulates early stages of human cerebellar development and model PCH with "cerebellar organoid" [9]. We will study the impact on the growth of the organoid, neuronal differentiation and apoptosis. Ultimately, we will perform RNAseq profiling experiments to gain further knowledge on the PRDM13-dependent transcriptional program during cerebellum development using both human and zebrafish cells.

Altogether this project will provide a comprehensive understanding of the neurodevelopmental defects caused by recently identified mutations causing PCH-type cerebellar malformations. Based on its role of and ability to recruit other chromatin modifying enzymes, the study of *PRMD13* can be an entry point to explore the epigenetic regulation of cerebellar development and could help to pinpoint other molecular defects affecting this program.

- **REFERENCES**

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EXPECTED PROFILE OF THE CANDIDATE

- **EXPERIENCE REQUIRED**
  We are looking for a highly motivated student with strong background in genetics and neuro-development. Basic molecular biology and cell culture experience is recommended.