Master Biologie Moléculaire et Cellulaire 'BMC',
Université Paris Cité - *UFR Sciences du Vivant*

Parcours : **Biologie et Développement Cellulaires 'BDC'**
https://master2bdc.ijm.fr/
Fiche de Projet de Stage de M2, 2026-2027

Unité INSERM UMR 1163 **Intitulé Equipe :** Genetics of Developmental Disorders **ED d'appartenance :** ED562 **Responsable de l'Equipe :** Pr J. AMIEL	**Responsables du Stage :** S. THOMAS & L. BOUTAUD Adresse : *Institut Imagine* - 24, Boulevard du Montparnasse - 75015 PARIS **Email** : sophie.thomas@inserm.fr ; lucile.boutaud@institutimagine.org **Tel :** 01 42 75 43 10

Titre du projet :
Dissecting the impact of *ASTN1* variants on human cortical development

Résumé du Projet de Stage (en 300 mots maximum, mots clés en gras)
Recent work has established ***ASTN1***, encoding astrotactin-1, as a causal gene for an **autosomal recessive neurodevelopmental disorder** characterized by developmental delay, epilepsy, and variable **cortical and cerebellar malformations**. These findings align with earlier studies in *Astn1*$^{-/-}$ mice, which display a cerebellar phenotype resulting from impaired neuronal migration and establish ASTN1 as a key regulator of **radial glia-guided neuronal migration**, a process required for the formation of laminar brain structures.

In parallel, high-throughput sequencing allowed us to identify *de novo* heterozygous missense variants in five patients with neurodevelopmental disorders, with or without cortical and cerebellar anomalies, suggesting that ASTN1-related disorders may not be restricted to a purely recessive model. We therefore hypothesize that distinct classes of *ASTN1* variants, biallelic loss-of-function versus heterozygous missense variants, potentially acting through gain-of-function or dominant-negative effects, lead to disease through different, yet potentially convergent, cellular mechanisms.

To investigate these mechanisms, the M2 student will leverage patient-derived and CRISPR/Cas9-engineered **induced pluripotent stem cell (iPSC) models** encompassing both variant types. We generated patient-derived iPSC lines with CRISPR/Cas9-corrected isogenic controls and complementary mutant lines derived from control iPSCs, enabling robust comparisons while controlling for genetic background and capturing human-specific developmental processes. These iPSC lines will be differentiated into **2D neural rosettes** and **3D cortical organoids** modeling human **cortical development**, recapitulating radial glia organization and neuronal migration.

Combined **immunofluorescence** and **confocal imaging**, together with **light-sheet microscopy**, will assess neural progenitor dynamics, neuronal differentiation, and cortical cytoarchitecture, with emphasis on radial glia scaffolding and neuronal migration. In parallel, **single-cell RNA sequencing** will characterize cellular composition and transcriptional states in mutant versus control organoids and identify transcriptional alterations associated with impaired neuronal migration and neuron–glia interactions.

Altogether, this project aims to dissect how *ASTN1* variants impair human cortical development and determine whether different mutational classes converge on common or distinct pathogenic pathways, providing broader insights into neuronal migration in the developing human brain.

Publications de l'équipe relatives au projet de stage

1. Boutaud L, et al**.** 2D and 3D Human Induced Pluripotent Stem Cell-Based Models to Dissect Primary Cilium Involvement during Neocortical Development. ***J Vis Exp***. 2022 Mar 25;(181). PMID: 35389978.
2. Guguin J, et al. A Taybi-Linder syndrome-related RTTN variant impedes neural rosette formation in human cortical organoids. ***PLoS Genet***. 2024 Dec 16;20(12):e1011517. PMID: 39680576.
3. Wiegering A, et al. A differential requirement for ciliary transition zone proteins in human and mouse neural progenitor fate specification. ***Nat Commun***. 2025 Apr 5;16(1):3258. PMID: 40188187.