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INTERNATIONAL PhD PROPOSAL

PhD Supervisor full name **Sophie THOMAS**

PhD PROPOSAL IDENTIFICATION

PhD Project title	Functional characterization of a novel gene for microcephaly
Project Acronym	EPICENTR
Project Keyword	Microcephaly, centrosome, primary cilium, histone acetylation

LABORATORY PRESENTATION

Laboratory Team Name	Embryology and genetics of malformations
Department IP	Institut Imagine INSERM UMR 1163
Doctoral school	BioSPC
University	Université de Paris
Laboratory website	https://www.institutimagine.org/en/users/sophie-thomas

PhD PROPOSAL

PhD Supervisor full name	Sophie THOMAS
PhD Supervisor position	Researcher
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PhD Proposal abstract (1000 characters maximum)

Microcephaly is a common cause of intellectual disability largely linked to the disruption of centrosomal proteins. Altered mitotic spindle formation/orientation has long been recognized as a mechanism underlying microcephaly, whereas the impact on the primary cilium (PC) has emerged only recently, highlighting the crucial roles of the PC during corticogenesis.
The project aims at functionally characterizing a novel microcephaly gene encoding a centrosomal protein that localizes to both the mitotic spindle poles and to the basal body of the PC and is also part of a histone-acetyl-transferase complex. Imaging and multiomics analyses on patient IPSC-derived cerebral organoids will improve our understanding of the roles of the PC during corticogenesis, especially in sensing and transducing key extracellular signals. This project also provides the opportunity to decipher the epigenetic mechanisms involving histone acetylation and regulating the size of the neocortex.

PhD Proposal (4000 characters maximum)

The cerebral cortex, which computes the high-level sensory, motor, and cognitive processes, has expanded dramatically during evolution. Corticogenesis is a complex and finely tuned developmental process that orchestrates the organization of many types of neurons through tight regulation of the survival, expansion, fate determination, and differentiation of neural stem and progenitor cells (NSPC). Disruption of any of the corticogenesis steps may result in anomalies of cortical development, encompassing a large spectrum of disorders representing a major cause of intellectual disability and which classification remains challenging. Among them, microcephaly is characterized by prenatal reduced brain size and has been largely linked to mutations in genes encoding proteins involved in the proliferation of NSPC, especially many centrosomal proteins. Centrosomes function as microtubule-organizing centers which form the spindle poles during mitosis while they nucleate a primary cilium (PC) in G0/G1 phase. The impact of centrosomal dysfunction on the mitotic spindle has long been considered a mechanism underlying microcephaly, whereas the consequences on PC dynamics and function have emerged only recently. PC are highly conserved microtubule-based organelles required for sensing and transducing various extracellular signals essential for coordinating diverse key processes of corticogenesis. In fact, they are present on all NSPC of the developing neocortex and are crucial for NSPC expansion, fate determination, neuronal migration, and maturation, highlighting the need for further delineation of the roles of the PC during corticogenesis.
Whole exome sequencing analysis allowed us to identify a novel candidate gene for microcephaly, encoding a centrosomal protein, in which *de novo* truncating mutations were identified in 6 patients from 5 distinct families. Beyond the strong genetic arguments and the fact that it encodes a centrosomal protein with potential consequences on both mitotic spindle and PC, this gene is an excellent candidate because of its high expression level in the ventricular zone of the developing human cerebral cortex. Moreover, this protein is part of a histone-acetyl-transferase complex, which raises a fundamental question about epigenetic mechanisms and their contribution to the regulation of neocortex expansion during evolution. To validate this gene as a microcephaly causing gene in human, the candidate will generate 2D and 3D IPSCs-based models of neocortical development, i.e. neural rosettes, isolated NSPCs and cerebral organoids, using described protocol established by the team (Boutaud 2022). For this purpose, blood cells from 3 patients have already been reprogrammed into IPSCs. In order to obtain isogenic controls, each IPSCs line will be edited by CRISPR/CAS9 to correct the mutations. Mutated and rescued IPSCs will be then used to generate complementary 2D and 3D cell-based models of cerebral development that will allow us to study the impact of the mutations on NSPC proliferation, survival and differentiation, on mitotic spindle formation and orientation, on PC biogenesis and function, including the transduction of PC-dependent signaling pathways. Thus, by combining cutting-edge imaging (lightsheet/confocal) and multiomics (ATAC-/CHIP-/RNA-seq) analyses on relevant cellular models, this project aims at dissecting the pathophysiological mechanisms underlying microcephaly associated with mutations of this novel candidate gene. Thus, beyond the obvious clinical impact on the molecular diagnosis of microcephaly for affected individuals and their families, this project will improve our understanding of the role of the PC during cerebral cortical development, especially in sensing and transducing key extracellular signals. This project also provides the opportunity to decipher the epigenetic mechanisms involving histone acetylation and regulating the size of the neocortex in human.

Expected profile of the candidate

The candidate shall have a solid background in genetics and/or neurosciences. Hands-on experience with induced pluripotent stem cell (IPSC) and/or with common techniques in molecular biology and/or fluorescence microscopy is required.