PhD PROJECT PROPOSAL

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Identifying evolutionary divergent functional gene modules in the human cerebellum development

Supervisor 1 (with name, email, affiliated laboratory and doctoral school affiliation)
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Abstract (10 lines)*

Speciation involves the emergence of new behavioral features that rely on the evolution of neural circuits. The human species displays higher cognitive features which have been linked in part to the evolution of the cerebral cortex. But the involvement of another brain region, the cerebellum, remains largely unexplored despite its association with both higher cognitive functions, neurodevelopmental disorders (e.g. autism spectrum) and motor disorders (e.g. cerebellar ataxia). Yet a description of its basic development and divergence at the cellular level in comparison with other species, including nonhuman primates, is lacking. The present project aims to identify, in humans, evolutionary divergent functional gene modules involved in the development and spatial organization of cerebellar neural circuits, at the cellular and circuit levels. Together, this project will uncover entirely new aspects of human cerebellar evolution and may lead to the identification of human species-specific sensitivity to brain disorders.

Keywords*
human brain evolution, human brain development, cerebellum, gene expression spatial pattern, human-specific duplicated genes.

Scientific question and Objectives (10 lines)*

The present project aims to identify, in humans, evolutionary divergent functional gene modules involved in the development and spatial organization of cerebellar neural circuits. In particular, spatial heterogeneity of Purkinje cells (cell intrinsic properties, synaptic innervation patterns, expression of biomarkers), the canonical cerebellar neuron and sole output of the cerebellar cortex, have been described in mouse to be key in the cerebellar computation properties. It is acquired during the postnatal development and its lost is linked to neurodegenerative disorders like cerebellar ataxia. However, the study of spatial heterogeneity and its development in humans, compared to nonhuman primates, have not been performed. The objectives of this project are: 1) to define the critical steps of the development of spatial heterogeneity of human Purkinje cells, 2) identify gene modules expressed specifically in humans compared to nonhuman primates, in time (development) and space (spatial heterogeneity), 3) functionally study the impact of one of such gene modules.

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Proposed approach (experimental / theoretical / computational) and research plan (20 lines)*

In the mouse cerebellum, Purkinje cells, the sole output of the cerebellum, display heterogeneous properties. For instance, they are organized in spatial stripes and within one stripe, Purkinje cells display differences in their cell intrinsic properties (neuronal excitability), synaptic innervation (from the excitatory and inhibitory inputs), which correlates with the expression of biomarkers, such as *AldoC* (encoding the zebrin-II protein). While all Purkinje cells are homogeneous in their expression of *AldoC* at birth, spatial heterogeneity (AldoC expression, difference in synaptic innervation) is acquired while the cerebellar circuits mature postnatally.

1) The PhD candidate will perform spatial transcriptomic using coppaFISH method on human, chimpanzee, marmoset and mouse cerebellar tissues, from birth to adulthood, and in different cerebellar regions (vermis, hemispheres, anterio-posterior axis). The list of genes (~100) will be defined by the candidate, based on known biomarkers of spatial heterogeneity (e.g. *AldoC*) and based on published cross-species snRNAseq datasets pointing to orthologous genes selectively expressed in the human cerebellum but for which spatial patterns have not been described. This will allow to enrich for divergent markers of spatial heterogeneity. The PhD candidate will also add human-specific genes, acquired through segmental duplication, and totally unexplored so far in the cerebellum in their expression and function.
2) The PhD candidate will develop algorithms to analyze the generated spatial transcriptomics dataset to then quantify and understand the development of spatial heterogeneity of cerebellar circuits. These quantifications will enable the automatic identification and characterization of the critical steps leading to that heterogeneity, the specificities of these heterogeneities in human compare to other primates and mammalians. It will finally help identifying the gene modules that correlate with these heterogeneities.
3) Then, the PhD candidate will test functionally the impact of functional gene modules divergent in humans, by expressing them in the mouse cerebellum (gain-of-function experiment) and look at their cellular (e.g. synaptic innervation, biomarker expression pattern) and developmental impact.

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Interdisciplinarity and Implication of the two labs (15 lines)*
(In this section the collaboration of the two laboratories will be explained in details to explain why the project cannot be conducted by one team alone)

The Team of Baptiste Libé-Philippot (wet team) explores the evolution of human neuron properties and development, by combining cross-species histological comparisons, gain-of-function experimental models in the mouse and using human cerebellar organoids. Baptiste Libé-Philippot opened its team at the IBDM in 2024, after a postdoc in neurobiology in the laboratory of Pierre Vanderhaeghen (VIB – KUL, Leuven, Belgium), studying experimentally the role of human-specific duplicate genes in the development and physiology of human cerebral cortex neurons. Its team already acquired or secured all the tissue specimen, ethical agreements, necessary for this project.

The team of Léo Guignard (dry team) is a group of computer scientists, physicists and biologists with a strong interested in biology in general and more specifically in embryonic development. We develop novel computational methods and models that allow the analysis of very large 3D movies of animal embryonic development (up to 2TB per movie). We work in close relationship with biologists to tailor our methods so that they help to address fundamental biological questions.

For further information about the lab you can visit guignardlab.com.

Specify with whom the person recruited will collaborate and on what aspects *

The person will collaborate with Stéphane Bugeon (Inmed) to design spatial transcriptomic probes and perform spatial transcriptomic data acquisition.

PhD student's expected profile*

We expect a PhD student candidate with a strong general background in biology and neurobiology; and with a lab experience in brain histology, molecular biology, mouse experimentation and with an interest in data analysis and modelisation. We look for a rigorous and creative candidate who knows to work autonomously and in a collaborative way in a team. The candidate needs to be able to communicate well in English.

Is this project the continuation of an existing project or an entirely new one?

It is an entirely new project.

In the case of an existing project, please explain the links between the two projects (5 lines)* NA

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Two to five references related to the project*

1) Libé-Philippot B, Polleux F, Vanderhaeghen P. (2024) If you please, draw me a neuron – linking evolutionary tinkering with human neuron evolution, Curr Opin Genet Dev. 2024 Oct 1;89:102260
2) Sepp M, Leiss K, Murat F, Okonechnikov K, Joshi P, Leushkin E, Spänig L, Mbengue N, Schneider C, Schmidt J, Trost N, Schauer M, Khaitovich P, Lisgo S, Palkovits M, Giere P, Kutscher LM, Anders S, Cardoso-Moreira M, Sarropoulos I, Pfister SM, Kaessmann H. (2024) Cellular development and evolution of the mammalian cerebellum. Nature. 2024 Jan;625(7996):788-796. doi: 10.1038/s41586-023-06884-x.
3) Haldipur P, Millen KJ, Aldinger KA. (2022) Human Cerebellar Development and Transcriptomics: Implications for Neurodevelopmental Disorders. Annu Rev Neurosci. 2022 Jul 8;45:515-531. doi: 10.1146/annurev-neuro-111020-091953. Epub 2022 Apr 19.
4) Bugeon S*, Duffield J, Dipoppa M, Ritoux A, Prankerd I, Nicoloutsopoulos D, Orme D, Shinn M, Peng H, Forrest H, Viduolyte A, Reddy CB, Isogai Y, Carandini M, Harris KD*. (2022) A transcriptomic axis predicts state modulation of cortical interneurons, Nature. 2022 Jul;607(7918):330-338.

Two main publications from each PI over the last 5 years*

- **Libé-Philippot B**, Lejeune A, Wierda K, Louros N, Erkol E, Vlaeminck I, Beckers S, Gaspariunaite V, Bilheu A, Konstantoulea K, Nyitrai H, De Vleeschouwer M, Vennekens KM, Vidal N, Bird TW, Soto DC, Jaspers T, Dewilde M, Dennis MY, Rousseau F, Comoletti D, Schymkowitz J, Theys T, de Wit J*, Vanderhaeghen P*. (2023) LRRC37B is a human modifier of voltage-gated sodium channels and axon excitability in cortical neurons, Cell 2023 Dec 21;186(26):5766-5783.e25

- **Libé-Philippot B**, Iwata R, Recupero AJ, Wierda K, Bernal Garcia S, Hammond L, van Benthem A, Limame R, Ditkowska M, Beckers S, Gaspariunaite V, Peze-Heidsieck E, Remans D, Charrier C, Theys T, Polleux F, Vanderhaeghen P*. (2024) Synaptic neoteny of human cortical neurons requires species-specific balancing of SRGAP2-SYNGAP1 cross-inhibition, Neuron 2024 Oct 9:S0896-6273(24)00645-7

- Abhishek Sampath Kumar, Luyi Tian, Adriano Bolondi, Amèlia Aragonés Hernández, Robert Stickels, Helene Kretzmer, Evan Murray, Lars Wittler, Maria Walther, Gabriel Barakat, Leah Haut, Yechiel Elkabetz, Evan Z. Macosko*, **Léo Guignard***, Fei Chen* & Alexander Meissner* Spatiotemporal transcriptomic maps of whole mouse embryos at the onset of organogenesis. Nat Genet 55, 1176–1185 (2023). https://doi.org/10.1038/s41588-023-01435-6

- Alice Gros, Jules Vanaret, Valentin Dunsing-Eichenauer, Agathe Rostan, Philippe Roudot, Pierre-François Lenne, **Léo Guignard***, Sham Tlili* (2025) A quantitative pipeline for whole-mount deep imaging and analysis of multi-layered organoids across scales. eLife 14

Project's illustrating image

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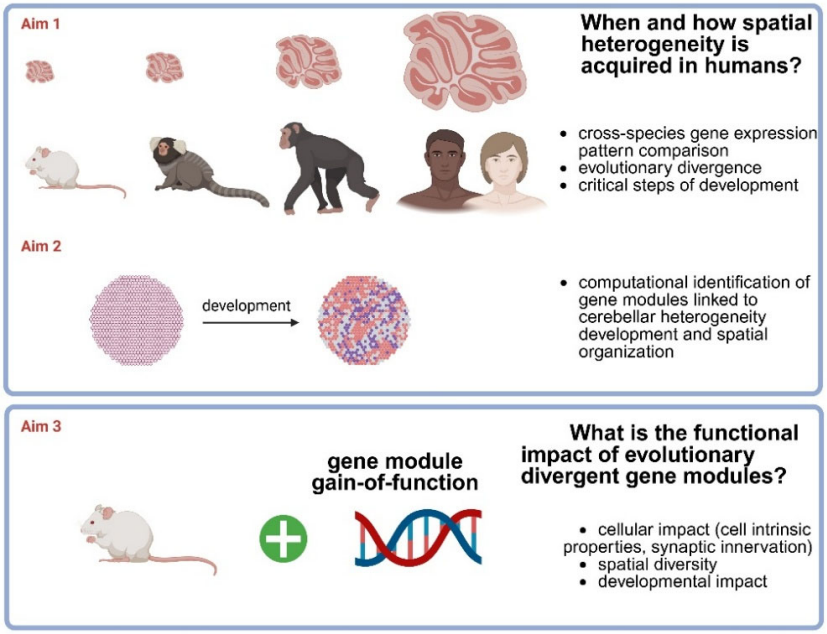

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